Biotechnology Advances 54 (2022) 107814

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

The Yin and Yang of exosome isolation methods: conventional practice, microfluidics, and commercial kits

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ARTICLE INFO

Keywords:
Microfluidics
Exosome
Isolation methods
Biomarker
Extracellular vesicles
Lab-on-a-chip
Commercial kits

ABSTRACT

Exosomes are a subset of extracellular vesicles released from various cells, and they can be found in different bodily fluids. Exosomes have been utilized as biomarkers to diagnose many diseases and to monitor therapy efficiency as they represent the status and origin of the cell, which they are released from. Considering that they co-exist in bodily fluids with other types of particles, their isolation still remains challenging since conventional methods are time-consuming, user-dependent, and result in low isolation yield. This review summarizes the conventional strategies and microfluidic-based methods for exosome isolation along with their strengths and limitations. In particular, microfluidic devices emerge as a promising approach to tackle the existing limitations of conventional methods, and they provide unique features, such as operating with minute volume of samples and rapid process, in order to isolate exosomes with the high yield and the high purity, which make them unprecedented tools for molecular biology and clinical applications in exosome research. This review further elaborates on the existing microfluidic-based exosome isolation methods and denotes their benefits and drawbacks. Herein, we also introduce various commercially available platforms and kits for exosome isolation along with their working principles.

1. Introduction

Extracellular vesicles (EVs) are released from nearly all cells and play an essential role in several physiological and pathological conditions (Théry et al., 2002). EVs are mainly categorized as exosomes, microvesicles, and apoptotic bodies considering their origin and size (Gardiner et al., 2016; van der Pol et al., 2012; Yáñez-Mó et al., 2015). Briefly, exosomes are tiny lipid bilayer vesicles (Melo et al., 2015)–they span from 30 to 150 nm in size and from 1.08 to 1.22 g/mL in density, and they are derived from multivesicular bodies (MVBs). According to prior reports in the literature, there are some discrepancies/variations in defining size and density ranges of exosomes (30-100 nm in size and 1.13-1.19 g/mL in density (Théry et al., 2002); 50-150 nm in size and 1.15-1.19 g/mL in density (Chiou and Ansel, 2016); and 30-150 nm in size and 1.08-1.22 g/mL in density (Samuel et al., 2017)). Considering the contents of exosomes, the lipid bilayer, in particuular, acts as a barrier to the protected internal environment, and it prevents the enzymatic degradation of exosomes (Enderle et al., 2015). While investigating the mechanism of exosome formation, we realize that after some maturation processes, endosomes formed by the invagination of cellular membrane generate multivesicular bodies, and exosomes are released after the formation of plasma membrane-fused multivesicular bodies (Farooqi et al., 2018; Vader et al., 2016; Vlassov et al., 2012). On the other hand, microvesicles with a density of 1.12 to 1.16 g/mL have a wide size ranging between 50 and 1000 nm, and they are formed through direct budding of outward plasma membrane. Apoptotic bodies ranging from 50 nm to 5,000 nm are secreted by apoptotic cells, and they are released during the late stages of programmed cell death. Overall, the biogenesis pathway of EVs is illustrated in **Fig. 1A**.

While focusing more on exosomes, they are present in several types of biofluids, including blood, urine, bile, bronchoalveolar lavage fluid (BALF), sputum, saliva, pleural fluid, bronchoalveolar lavage fluid, cerebrospinal fluid (CSF), vitreous, ascites, and breast milk, given their small size and ability to spread (Admyre et al., 2007; Barile and Vassalli, 2017; Gonzales et al., 2009; Kalra et al., 2013; Keller et al., 2011; Runz et al., 2007; Stuendl et al., 2016; Torregrosa Paredes et al., 2014; Zhao et al., 2018a, 2018b; Zhu et al., 2014). Back when EVs were discovered for the first time, these vesicles including exosomes were regarded as cell

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https://doi.org/10.1016/j.biotechadv.2021.107814
Received 2 May 2021; Received in revised form 8 August 2021; Accepted 8 August 2021
Available online 11 August 2021

debris (cellular by-product) and were not thoroughly studied (Johnstone et al., 1987; Raposo et al., 1996). However, it is now explicitly presented that exosomes contain their specific biomarkers, including CD9, CD81, other proteins (transcription factors and oncogenic regulators), and genes from their parental cells, such as microRNAs, lncRNAs, and circleRNAs. By activating cell surface receptors, exosomes can transfer their internal contents into the acceptor cell (Valadi et al., 2007). In addition, exosomes play pivotal roles in cell renewal, immune surveillance (Théry et al., 2009), tissue repair (Gatti et al., 2011), blood coagulation (Colombo et al., 2013; del Conde et al., 2005), and intercellular communication. These vesicles encapsulate biomolecules, including proteins, lipids, and nucleic acids (Fonseca et al., 2016; Lee et al., 2012; Tan et al., 2018), with pathological and physiological blueprints from their parental cells (**Fig. 1B**).

Moreover, exosomes are considered as biomarkers in clinical applications for diagnosis and therapeutic applications (Chen et al., 2013; Lai et al., 2013; Patel et al., 2018). In particular, exosomes have shown to be involved in cancer progression, including tumor formation, proliferation, metastasis, and drug resistance, by changing the tumor microenvironment (Al-Nedawi et al., 2008; Chen et al., 2018; Hoshino et al., 2015; Liang et al., 2017a, 2017b, 2017c; Luga et al., 2012; Nabet et al., 2017; Ruivo et al., 2017; Yu et al., 2015). Researchers have demonstrated that the number of exosomes released by tumor cells is higher than those in normal cells, and hence, it would be meaningful to analyze exosomes extracted from cancer cells (Whiteside, 2016). In a nutshell, exosome research is therefore considered to be a promising path for cancer research (An et al., 2017; Lagoa et al., 2020; Sreepadmanabh and Toley, 2018; Zhao et al., 2018a, 2018b). It has been shown that exosomal nucleic acid differs not only in healthy individuals and in people with cancer (Tang et al., 2013; Zhan et al., 2018), but also in the different stages of the disease (Li et al., 2019). Accordingly, due to the specificity of the exosomal nucleic acids under different conditions, they can be utilized as biomarkers to identify and manage cancer at various stages. For instance, Dong *et al.* have shown that exosomal mRNAs and lncRNAs in blood samples could be used as biomarkers for colorectal cancer detection. Moreover, it concluded that since exosomes have far higher RNA levels compared to apoptotic bodies and microvesicles, they are a promising tool for cancer diagnosis (Dong et al., 2016). Exosomes, as previously stated, can be used not only for diagnostic purposes, but also for therapeutic applications. In particular, Kamerkar *et al.* compared the effects of exosomes and liposomes in treating oncogenic KRAS in pancreatic cancer (Kamerkar et al., 2017). In this study, the engineered exosomes exhibited better RNA interference delivery to specific targets, and ultimately, suppressed cancer. As observed here, using biomarkers collected from a bodily fluid was more beneficial than traditional tissue collection samples and invasive methods. However, the main drawback of using biomarkers is that the samples usually contain soluble protein and aggregates, which causes contamination issues during the exosome isolation.

2. Exosome isolation methods

A variety of exosome isolation methods has been introduced, reliant on different principles (**Fig. 2**). Typically, these methods can be categorized as conventional methods and emerging methods. Although researchers have widely utilized conventional methods, their recovery yields and efficiencies are relatively low, as well as the fact that they need for a longer processing time. On the other hand, microfluidic-based methods, as one of the emerging techniques, show excellent capabilities in lower sample volume, rapid separation, high sensitivity, and higher separation yield. In this review, we first overview conventional techniques, and then, discuss microfluidic technologies comprehensively in the topic of exosome separation from various types of bodily fluids through physical and chemical features of exosomes. In addition, the limitations and advantages of each technique will be demonstrated explicitly to provide a broad perspective for future research directions. Moreover, this review provides new insight for exosome isolation methods and distinguishes the methods according to their working principle. By exhibiting several examples for each category, different methods are evaluated based on their working principle, the required sample volume, and the type of sample. Finally, we conclude the review with comprehensive research on the commercially available exosome isolation kits, platforms, and reagents.

2.1. Traditional exosome isolation methods

There are various types of conventional exosome isolation techniques, including differential centrifugation, density gradient ultracentrifugation, precipitation-based, immunoaffinity, ultrafiltration, and size-exclusion chromatography. The working principle, advantages, and disadvantages of different techniques are provided in **Table 1**. In this section, we briefly describe each method and provide several examples for each of them.

2.1.1. Differential Centrifugation

The DC is the gold standard and most widely-implemented approach for exosome extraction through low-speed to high-speed centrifugation of cells, cell debris, proteins, and vesicles (Jeppesen et al., 2014; Johnstone et al., 1987; Livshits et al., 2015; Momen-Heravi et al., 2013). The centrifugation speed of this method usually starts with $300 - 2000 \times g$

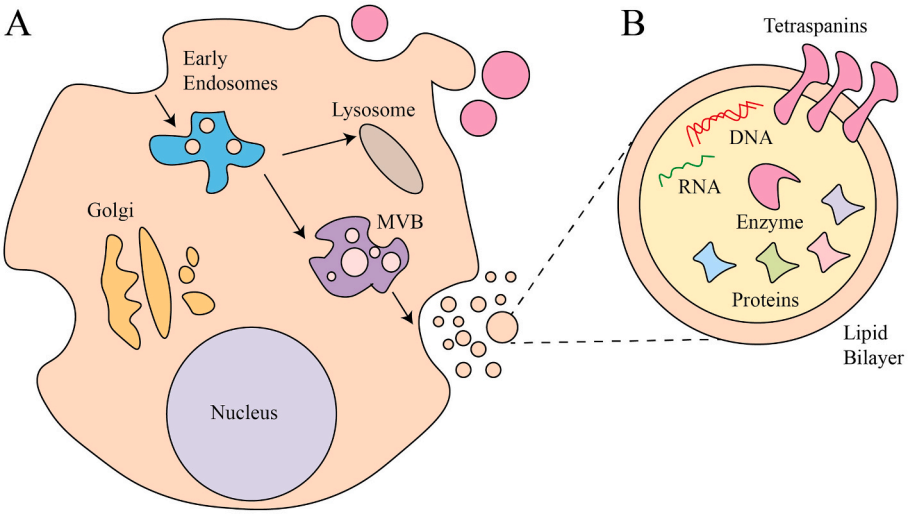

Fig. 1. (A) The biogenesis pathway of extracellular vesicles. Exosomes are formed by the inward budding of the cell plasma membrane, which produces multivesicular endosomes containing exosomes. Exosomes will be then released as a result of the fusion of multivesicular bodies and the cell plasma membrane. Microvesicles are formed through outward budding of the cell plasma membrane whereas apoptotic bodies are generated during cell death. (B) Exosome content. Exosomes mainly contain proteins, lipids, and nucleic acids.

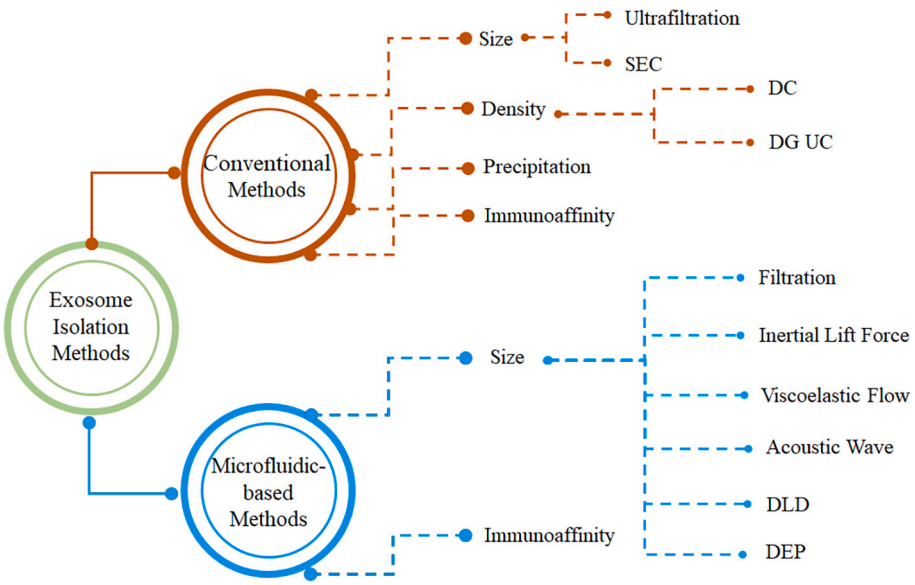

Fig. 2. Various exosome isolation methods (SEC: Size-Exclusion Chromatography; DC: Differential Centrifugation; DG UC: Density Gradient Ultracentrifugation; DLD: Deterministic Lateral Displacement; DEP: Dielectrophoretic).

for extracting cells, apoptotic bodies, and cell debris as pellets. Afterward, by increasing the speed up to 5000 – 10000 × g, larger EVs and protein pellets separated as precipitations based on their size and density. Finally, the supernatant will be exposed to a higher force (100000 × g) for 1-3 hours to separate exosomes. All centrifugation steps are operated at 4 ° C (Szatanek et al., 2015). In this step, the supernatant containing small proteins is discarded, and the pellets containing exosomes and contaminated proteins are washed and resuspended with phosphate buffered saline (PBS). Again, the solution is exposed to ultracentrifugation with a speed of 100000 × g for around an hour, and eventually, this step enables to produce a pellet that contains exosomes. It is worth mentioning that there is no unified approach for centrifugation, so that the number of steps, corresponding duration and speed can differ from one to another procedure (**Fig. 3A**) (Musante et al., 2013).

Moreover, considering that a significant number of vesicles may be lost during this process, DC is appropriate for experiments involving a considerable amount of the initial sample (Witwer et al., 2013). Hence, it can be concluded that this technique is relatively ineffective for a small amount of sample due to its low yield and purity. Besides, due to consecutive centrifugation steps, it is possible that the final exosome pellet contaminated by cell debris and apoptotic bodies remained from previous steps (Livshits et al., 2015). Although researchers have widely used this method, this technique is time-consuming and instrument-dependent (L.-G. Liang et al., 2017a, 2017b, 2017c; Merchant et al., 2017; Taylor and Shah, 2015). This method, on the other hand, has some benefits, including easy operation, low separation reagent contamination, acquisition of large exosomes, and low-cost per assay (not including the instrument cost).

2.1.2. *Density Gradient Ultracentrifugation*

Density gradient centrifugation (DG UC) is a modified version of the DC method for exosome isolation to obtain a higher purity by changing the surrounding medium to reduce the density from bottom to top (**Fig. 3B**). The vast majority of DC steps are also carried out herein. In this method, the sample is introduced to a gradient medium to create layers of different densities. By starting ultracentrifugation with a particular force, the particles in the sample move toward the gradient layers and will be located where their densities match the surrounding solution. The exosomes can be then separated from other vesicles with different densities by differential fraction collection (Kamerkar et al., 2017; Li et al., 2017a, 2017b; Vergauwen et al., 2017). Among different types of medium used for gradient generation, sucrose and iodixanol (OptiPrep) (Kalra et al., 2013) are the most widely applied medium in the literature to create discontinuous gradients (Van Veldhoven et al., 1996). Although some researchers showed that high exosome purity could be obtained using sucrose due to its non-toxic characteristics and neutral pH (Zeringer et al., 2015), several studies have illustrated that iodixanol has some advantages over the sucrose method. To delineate, it not only can the high viscosity and hyperosmotic properties of sucrose solution harm the exosomes (Neves et al., 2009), but it also takes longer to sediment the exosomes (Ford et al., 1994). On the other hand, since the iodixanol produces an isosmotic solution in different densities, the shape and size of vesicles can be better preserved when moving through the gradient (Cantin et al., 2008; Dettenhofer and Yu, 1999; Van Deun et al., 2014). In particular, given the exosome contamination with retroviruses *in vitro* experiments, although it is necessary to separate them, it will not be facile due to their similarity in size and density. Specifically, Cantin *et al.* reported that a sucrose solution was not an effective way to isolate HIV-1 from exosomes, but they could be separated to the various sedimentation velocities in the iodixanol gradient (Cantin et al., 2008). Iwai *et al.* successfully implemented the iodixanol DG UC method to isolate exosomes from human saliva. According to the results, the isolated EVs had a smaller diameter and higher density than those in conditioned cell media (Iwai et al., 2016).

Considering the capacity of the gradient levels, only a small amount of sample can proceed with this method (Lane et al., 2015). It takes more time to reach equilibrium at each gradient level in order to achieve high yield isolation. According to Li *et al.*, the process took around 16 to 90 hours to obtain appropriate separation that was a way longer than that of DC (Li et al., 2017a, 2017b). Co-isolation of different particles with similar density is also another issue in this method. Yuana *et al.* utilized the DG UC method to isolate high-density lipoproteins (HDL), low-density lipoprotein (LDL), and very low-density lipoprotein (VLDL) from human plasma. Here, the co-precipitation of EVs and HDL was observed since they shared similar densities (Yuana et al., 2014). In another study, even though LDL has a lower density than exosomes, co-isolation of exosomes and LDL has occurred (Sódar et al., 2016). On the other hand, researchers showed that the iodixanol DG method produced a pure population of exosomes (Kalra et al., 2013). Lobb *et al.* compared various exosome isolation techniques, such as size exclusion chromatography, qEV columns, and the DG method. In order to assess the purity

Table 1
Conventional exosome isolation methods.

Method	Working principle	Advantages	Disadvantages	Reference
Differential Centrifugation (Sequential Ultracentrifugation)	• Size-based separation under alternative low/high centrifugation speed	• Low cost • Simple operation • Suitable for large volume preparation • No additional reagents	• Potential damage to exosomes • Low portability • Time-consuming and user dependent • Not suitable for small amount of sample • Specific equipment requirement • Low yield	(Lee et al., 2019, 2018, 2016)
Density Gradient Ultracentrifugation	• Density-based separation method • Combination of ultracentrifugation and density gradient medium	• High purity • Simple operation	• Potential damage to exosomes • Low portability • Time-consuming • Sensitive to centrifugation time • Low Yield	(Cantin et al., 2008; Jeurissen et al., 2017; Onódi et al., 2018)
Size-Exclusion Chromatography	• Size-based separation • Polymer column filled with nanoporous beads	• High purity • Intact structure of isolated exosomes • Capable of processing various types of samples • Good reproducibility	• Time-consuming • Low recovery • Relatively expensive and complex device • Low sample volume	(Liu et al., 2020; Ludwig et al., 2019; Oeyen et al., 2018)
Ultrafiltration	• Size-based separation • Trapping exosomes based on their size in a nanomembrane	• Simple operation • Portability • Fast procedure • Capable of operating with low amount of sample • Low equipment cost • Suitable for large volume preparation	• Membrane clogging • Possible damage induced by shear stress • Moderate purity	(Cooper et al., 2020; Lobb et al., 2015; Tauro et al., 2012)
Precipitation Methods	• Size and density-based separation • Using polymers alters the exosome solubility in the solution	• High throughput • Simple operation	• Low purity due to the contamination with polymers • Pre and post cleanup steps required • Time-consuming • Contaminants hinder downstream	(Chung et al., 2020; Rider et al., 2016; Ryu et al., 2020)
Immunoaffinity	• Antibodies employed for capturing exosomes	• High purity and selectivity • Specific exosomes isolation	• Non-specific binding • Costly • Low processing volume and yield • Exosomes attached to the beads • Exosome elution steps required	(Kowal et al., 2016; Sharma et al., 2018a, 2018b; Song et al., 2021)

of the isolated exosomes, exosome markers were utilized so that the number of particles per μg of the provided proteins was considered a good indicator of exosome purity. As reported in this study, the iodixanol DG method yielded the purest exosomes (Lobb et al., 2015). Furthermore, compared to the ultracentrifugation method, the iodixanol gradient improved the purity in the exosome isolation (Van Deun et al., 2014).

2.1.3. Size-Exclusion Chromatography

Size-Exclusion Chromatography (SEC) (gel filtration), another size-based isolation approach (Böing et al., 2014; Taylor and Shah, 2015), utilizes a stationary phase consisting of polymeric porous beads with a particular pore size, which separates particles with different radii (Fekete et al., 2014; Hong et al., 2012). Based on the particle and pore size, smaller particles can penetrate the pores, leading to later elusion, whereas larger components cannot diffuse into the pores and move between the porous beads (**Fig. 3C**). Some SEC-based products, including qEV (iZON Science) separation columns, PURE-EVs (Hansa Biomed), and EVSecond (GL Sciences) purification columns for exosome purification, are commercially available. Another technology that takes advantage of both precipitation and SEC approach is the Exo-spin (Cell Guidance System). Baranyai *et al.* drew a comparison in terms of purity and yield between UC and SEC methods for exosome isolation. While both UC and SEC techniques exhibited low efficiency in this study, lower albumin amounts were observed using the SEC method, pointing out that this method provided high purity in the exosome isolation (Baranyai et al., 2015). This method has many other benefits, including ease-of-use, low-cost, preservation of original exosomes structure and morphology, and obtaining uniform size of exosomes (Taylor et al., 2011). However, SEC is mostly hindered by the passing of similar size particles, lengthy turnaround time, and limited processing sample volume, hence requiring further purification steps (Böing et al., 2014).

2.1.4. Anion Exchange Chromatography

Anion exchange chromatography (AIEX) method is another chromatography-based technique, reliant on the opposite charge attraction. Once the negative charge on the surface of EVs is bound to the positively-charged chromatography column (Deregibus et al., 2016),

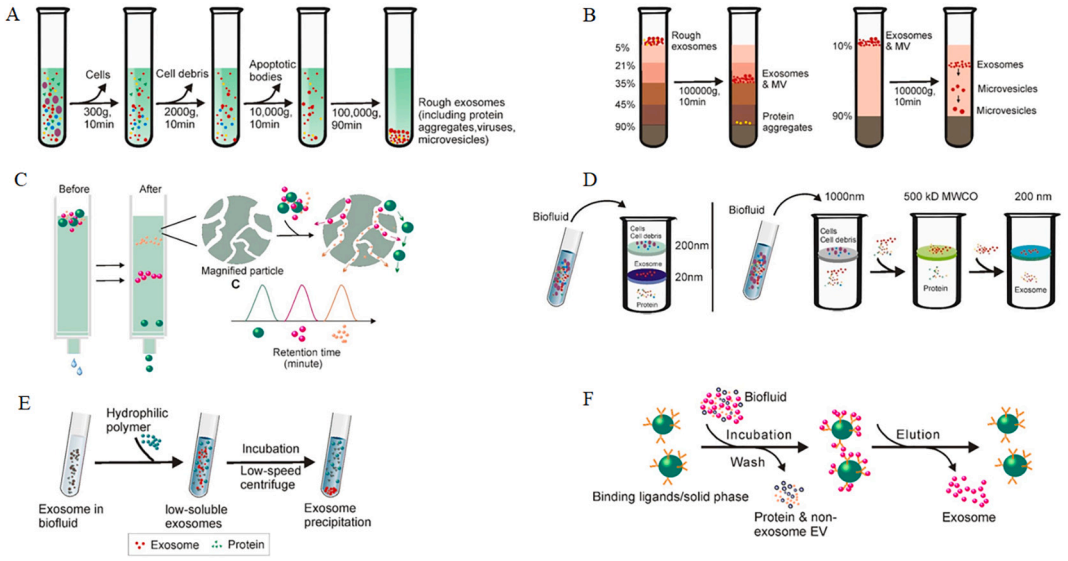

Fig. 3. Conventional exosome isolation methods. (A) Differential ultracentrifugation, (B) Gradient density ultracentrifugation, (C) Size-exclusion chromatography, (D) Ultrafiltration, (E) Precipitation-based method, (F) Immunoaffinity-based method. Reused with permission from Ref. (Yang et al., 2020). Copyright 2020, Ivyspring International Publisher.

they can be detached by adjusting the mobile phase's ionic strength. Anion exchange monolith matrices, for instance, were used by Zaveckas *et al.* to separate PCV2 Cap virus-like particles (VLPs) from yeast lysates (Zaveckas et al., 2015). Kim *et al.* utilized a low-speed centrifugal technique followed by an AEIX to isolate EVs produced by mesenchymal stem cells (Kim et al., 2016). Heath *et al.* compared the efficiency of different isolation techniques, including AEIX, tangential flow filtration, and ultracentrifugation for HEK293T cell-derived EVs isolation (Heath et al., 2018). Although AIEX and UC methods have revealed similar yield and purity, AIEX is a single-step method that can readily be scaled up and employed for large-scale clinical applications (Heath et al., 2018). This method could also isolate EVs in cell culture supernatant and biological samples (Popovic et al., 2018).

2.1.5. Ultrafiltration

It is also possible to separate exosomes based on the difference in size between different particles (Lobb et al., 2015; Tauro et al., 2012). In this strategy, membranes of different pore sizes are utilized to separate particles of different sizes (Vu et al., 2018). Considering the size of exosomes, this method can distinguish them from larger particles (Wang et al., 2013). During filtration, larger particles cannot pass through the membrane filter, whereas smaller particles can pass through the membrane pore size (**Fig. 3D**). Exosomes experience relatively high pressure during the passage of the membrane pores, which can damage the exosomes, change their morphology, and potentially interfere with downstream analysis (Greening et al., 2015). Moreover, smaller particles trapped in the membrane can cause the clogging issue and prevent the desired particle from crossing the membrane, resulting in a lower recovery rate (Cheruvanky et al., 2007; Vergauwen et al., 2017). Additionally, the purity of the isolated exosomes using this method is relatively low since the particles with a similar size of exosomes can also pass through the membrane. Another parameter that can impact the ultrafiltration process is the filter material. By considering various types of filters, Vergauwen *et al.* showed that the regenerated cellulose was the most efficient material for exosome separation from urine and plasma samples (Vergauwen et al., 2017).

Researchers have recently utilized sequential filtration that demonstrates higher efficiency than single-step filtration (Heinemann et al., 2014; Heinemann and Vykoukal, 2017; Li et al., 2017a, 2017b). In this process, the sample passes through membranes of different sizes. Larger sample components, including cells and cell debris, are separated first, and then, by lowering the membrane pore sizes in sequential steps, smaller components can be separated. In this procedure, lower pressure is applied to the exosomes to preserve their original shape and functionality. In addition, a sample with different cut-offs can be separated, and high purity exosomes can be obtained and used for further analyses.

2.1.6. Precipitation-based methods

The precipitation-based techniques are rapid and effective methods for exosome purification that contains the incubation of precipitation agents and samples, followed by the low-speed centrifugation or filtration steps (**Fig. 3E**) (Brown and Yin, 2017). Either salt solutions (Brownlee et al., 2014) or polymers, commonly polyethylene glycol (PEG) (Yamamoto et al., 1970), can be utilized as the precipitation agents. At first glance, the solution is saturated by dissolving polymers, allowing exosomes to be less soluble and precipitate. Having precipitated, further low-speed centrifugation (1500×g) or filtration will separate the exosomes (Weng et al., 2016; Zeringer et al., 2015). The produced pellet will then be washed with PBS for the following downstream analysis. PEG, in particular, has garnered attention among different polymers due to its unique characteristics, including low-cost, hydrophilicity, and easy precipitation procedures. Despite the facile use of this method, the key drawback of the precipitation process is the interference of a precipitation agent and membrane fusion of EVs (Posokhov and Kyrychenko, 2013). Co-precipitation of non-exosomal particles, including protein and polymeric substances, which leads to less effective exosome isolation, is also a downside of this process (Zarovni et al., 2015).

Mentioning the recent literature, Weng *et al.* utilized high-resolution electron microscopy to demonstrate the size and shape of the extracted exosome aggregates and unveiled the mechanism of the PEG-based precipitation strategy in detail (Weng et al., 2016). As a note, the pH value of isolation environment is one of the critical parameters that strongly hinders the isolation yield. Ban *et al.* reported that the highest exosome isolation yield was obtained in acidic environment, yet the isolation yield decreased as the pH value increased (Ban et al., 2015). To maximize EV isolation yield, Brennan *et al.* compared various techniques of separation, including polymer precipitation, ultracentrifugation, size exclusion chromatography, and DG UC, and observed the highest number of EVs collected from human serum through the polymer precipitation technique (Brennan et al., 2020).

Another precipitation-based approach for isolating EVs is protein-

organic solvent precipitation (PROSPR) (Gallart-Palau et al., 2016). The basic principle of this method is that with organic solvents, including acetone, chloroform, and trichloroacetic acid (TCA), a soluble protein is precipitated such that the EVs remain in the supernatant. After protein removal, the supernatant-containing EVs is concentrated by filtration or vacuum concentrators (Gallart-Palau et al., 2015).

Charge-based precipitation is also introduced as one of the efficient isolation methods. Considering that EVs are negatively charged particles (Grant et al., 2011; Keller et al., 2009; Taylor et al., 2014), they can interact with positively charged particles, mainly protamine, to isolate EVs in plasma, cell culture, and saliva samples. Deregibus *et al.* showed that the combined use of PEG and protamine reveals higher isolation yield rather than the use of them separately (Deregibus et al., 2016). This method benefits from its low-cost, simplicity, and obtaining of intact EVs like precipitation as mentioned in earlier techniques. However, not only is further gel filtration required, but protamine residue may also contaminate the final target. The charge neutralization, another alternative approach based on precipitation strategy, was proposed (Brownlee et al., 2014), and accordingly, acetate was used in this work to precipitate EVs through neutralizing negatively charged EVs with acetate.

2.1.7. Immunoaffinity capture-based isolation

Immunoaffinity method works based on the interactions of antibody and antigen (Mincheva-Nilsson et al., 2016). Generally, exosomes have specific antigens on their surfaces which can be targeted by specific antibodies. In this way, it is possible to capture the exosomes in the sample when particular antibodies are used (Gholizadeh et al., 2017; Pariset et al., 2017; Tauro et al., 2013) (**Fig. 3F**). Among various well-known types of biomarkers, the most common biomarkers used for exosome isolation are tetraspanins, including CD9, CD63, CD81, CD82, and CD151 (Clayton et al., 2001; Li et al., 2017a, 2017b). For instance, the specific antibodies are immobilized on the different surfaces, including magnetic beads, plates, chromatography matrices, and microfluidic devices to capture exosomes on the surface (Chen et al., 2010; Li et al., 2020a, 2020b; Théry et al., 2009; Zeringer et al., 2015). Likewise, there are specific biomarkers for microvesicle isolation which are distinct from that of exosomes. In particular, it has been shown that Annexin A1, which is shed from plasma membranes, can be used for microvesicle isolation while undetectable in exosomes (Jeppesen et al., 2019).

Immunoaffinity chromatography-based purification methods rely on separating a particular component from a mixture due to its affinity to bind to a specific antibody. In this scenario, antibodies are immobilized in the stationary phase, and the sample flow passes it in a mobile phase, similar to conventional chromatography separation methods that require two stationary and mobile phases. Since the sample has different components with different affinities to the immobilized antibodies, each component's elution rate varies. Therefore, the sample is attached to the surface when the remainder of the sample is first eluted. Using mass spectrometry, the targeted sample can be eluted and used for further analysis as well (Cutler, 2004).

Greening *et al.*, for instance, compared various methods, including UC, immunoaffinity methods, and DG UC, and the immunoaffinity method provided the highest efficiency (Greening et al., 2015). Moreover, Tauro *et al.* investigated the efficiency of ultracentrifugation, DG UC, and immunoaffinity methods using the anti-EpCAM-coated magnetic beads technique for exosome separation, and likewise, the immunoaffinity method provided the highest yield (Tauro et al., 2012). However, the feasibility of this method, despite its high efficiency, depends heavily on the accessibility of particular antibodies to capture specific exosomes. It is, therefore, essential to identify different types of exosomes and to find new antibody-antigen pairs. In particular, Tauro *et al.* examined the possibility of capturing two populations of exosomes from human colon carcinoma cells (Tauro et al., 2013). Targeting the A33 and EpCAM surface markers, exosomes were isolated in this study *via* magnetic beads. In another study, melanoma-derived exosomes in plasma were captured with a monoclonal anti-chondroitin sulfate peptidoglycan (CSPG4) antibody using streptavidin-coated magnetic beads (Sharma et al., 2018a, 2018b). While this method offered high selectivity to provide high purity, the drawbacks on non-specific binding restricted its utility (Konoshenko et al., 2018).

2.2. Microfluidic-based exosome isolation techniques

Recently, microfluidic-based platformed have been adapted to denote applications in the field of disease diagnosis, treatment, and other biological applications (Deshmukh et al., 2020; Guo et al., 2021; Inan et al., 2017; Inci et al., 2021, 2018, 2015; Nath et al., 2020). The inherent characteristics of the microfluidic devices, including high surface-to-volume ratio, low sample consumption, low analysis time, laminar flow, and ease-of-use, make them suitable for exosome isolation with a high recovery rate and purity for clinical applications (Asghar et al., 2016; Mani et al., 2016; Marzano et al., 2020; Park et al., 2020). Although there are various types of materials used in microfluidic device fabrication, including glass (Zeibi Shirejini and Mohammadi, 2017), paper (Hu et al., 2016), silicon (Zhuo et al., 2020), polydimethylsiloxane (PDMS) (Torino et al., 2018), polymethyl methacrylate (PMMA) (Inci et al., 2020), and metals (Stainless et al., 2019), PDMS is the most frequently employed material due to its key characteristics such as transparency, biocompatibility, cost-effectiveness, and flexibility (Ayoib et al., 2020). Microfluidic devices have various components including micromixers, valves, microchannels, and pumps (Fernandes and Krühne, 2018). In particular, it has been shown that microfluidic devices are able to isolate exosomes in various samples with high selectivities and yields while lowering the processing time, cost, and sample consumption compared with conventional isolation techniques (Guo et al., 2018). Generally, microfluidic techniques can be classified as either passive or active. The active methods require external actuators, such as electric fields, magnetic fields, and acoustic waves. On the other hand, passive methods do not need any external fields for particle sorting (Salafi et al., 2017). Although active separation techniques improve the exosome isolation throughput and efficiency, it increases both complexity of the system and the cost needed to run the process. Examples of various microfluidic-based isolation methods along with their highlights and limitations are presented in **Table 2**.

2.2.1. Filtration

As elaborated earlier sections, filtration is a commonly used label-free isolation and separation process, and it is also adapted to the microfluidics that utilizes microchannel-integrated porous membranes or nanoarrays to collect exosomes corresponding to their size (L. G. Liang et al., 2017a, 2017b, 2017c). Typically, there are two types of filtration operations employing dead-end (L.-G. Liang et al., 2017a, 2017b, 2017c; Li et al., 2017a, 2017b; Woo et al., 2017) and cross-flow (tangential-flow) (Busatto et al., 2018; Heath et al., 2018; Kim et al., 2021) membranes. In the dead-end process, all samples are perpendicularly introduced to the surface of the membrane, resulting in the accumulation of the rejected particles across the surface of the membrane. After a while, the thickness of the rejected particles layer increases and not only does pressure drop increase, but the quality of the permeate decreases. On the other hand, in cross-flow operations, the sample passes along with the membrane surface, and hence, the clogging is lower compared to the dead-end membrane configuration. Compared with the cross-flow, the main advantage of the dead-end method is the high recovery. Therefore, a combination of both methods, which benefits from the advantage of both techniques, ideally results in a high recovery and lower clogging tendency. This combination method is called a hybrid approach (Mulder and Mulder, 1996). In this process, the sample is injected from one side to the membrane, and after a while, from the opposite side, the sample is introduced to the membrane to back-flush the filter. This cycle is repeated, and high recovery and lower

Table 2
Microfluidic exosome isolation methods.

Method	Working Principle	Advantages	Disadvantages	Sample	Sample Volume	Reference
Filtration		• Simple assembly • Simple operation	• Complicated fabrication process • Clogging	Urine	8000 µl	(Liang et al., 2017a, 2017b, 2017c)
Inertial lift force		• High-throughput	• Co-isolation of small particles	Blood	NA	(Dudani et al., 2015)
Viscoelastic flow		• Simple operation • High-throughput	• Reagent addition	Serum	100 µl	(Liu et al., 2017a, 2017b)
Acoustic waves	Size based separation	• High-throughput • High efficiency	• Required external force	Whole blood	1500 µl	(Wu et al., 2017)
Dielectrophoretic		• High-throughput	• Required external force • Low purity • Fluid conductivity requirements	Undiluted human plasma	30-50 µl	(Ibsen et al., 2017)
Deterministic Lateral Displacement		• No external force and additive	• Challenging pillar array fabrication • Low-throughput • Clogging • High cost	Urine	90 µl	(Wunsch et al., 2016)
Affinity-based exosome isolation methods	Based on specific binding of exosomes and immobilized antibodies	• Specific exosome separation • High purity	• Instability of the antibodies when exposed to the buffer solution • Isolating only specific exosomes that have target antigens on their surface	Plasma	30 µl	(He et al., 2014)

fouling can occur. Woo *et al.* took the advantage of this idea to combine centrifugation and nanofiltration processes, and consequently, they isolated exosomes from different samples, *i.e.,* cell-culture supernatant (CCS) and urine samples from bladder cancer patients (Woo et al., 2017). This technique relied on the integration of a lab-on-a-disc device and two nano filters, *i.e.,* Exodisc, and the researchers also benchmarked

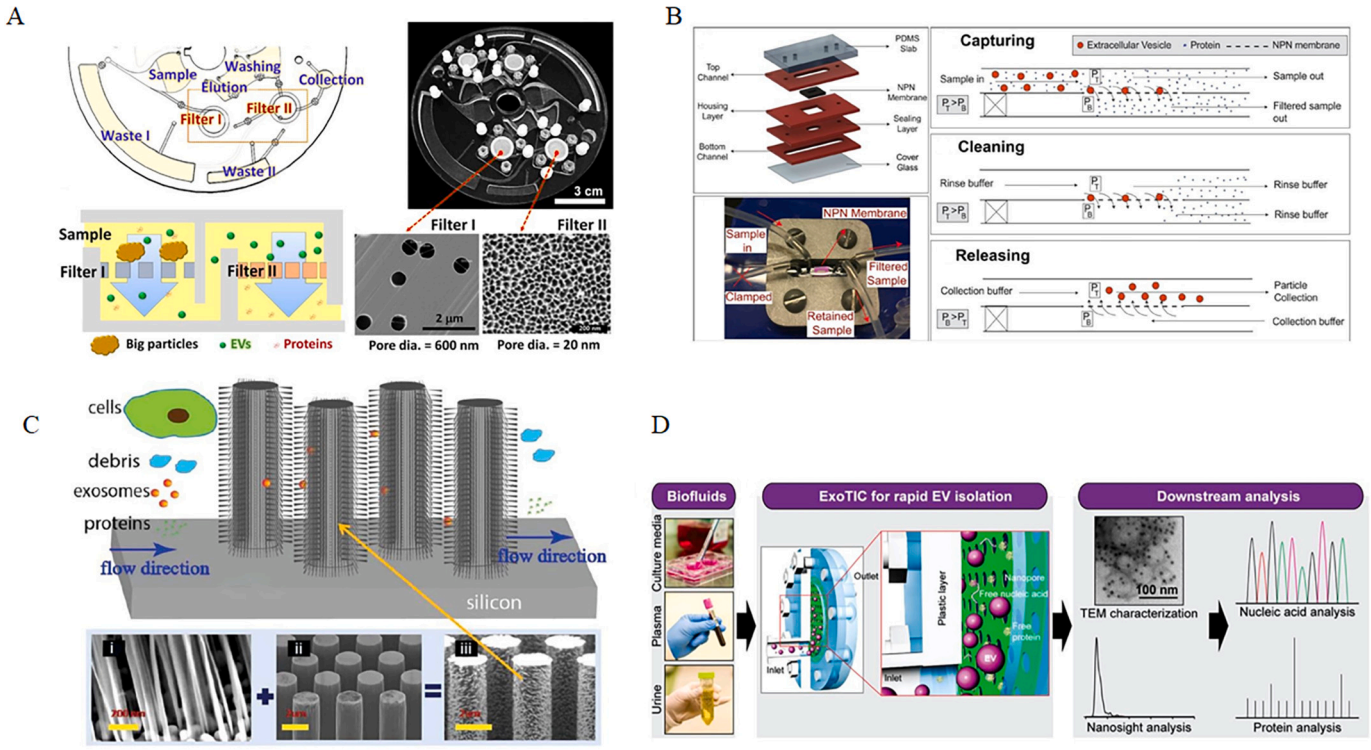

Fig. 4. Examples of microfluidic devices for exosome separation using the filtration strategies. (A) The schematic presents the isolation of extracellular vesicles on a microfluidic device using a dead-end filtration system with two filters. Reused with permission from Ref. (Woo et al., 2017). Copyright 2017, American Chemical Society. (B) The representative schematic states extracellular vesicle isolation through the tangential membrane system. Reused with permission from Ref. (Dehghani et al., 2019). Copyright 2019, Wiley. (C) Ciliated micropillar structure coated with nanowires is demonstrated. Cell and cell debris cannot pass the micropillar array due to the distance between micropillars while exosomes are enriched in by trapping in nanowires. The inset images present (i) porous silicon nanowire; (ii) micropillars; (iii) ciliated micropillars. Reused with permission from Ref. (Wang et al., 2013). Copyright 2013, Royal Society of Chemistry. (D) The workflow and schematic of ExoTIC device for exosome isolation. Reused with permission from Ref. (Liu et al., 2017a, 2017b). Copyright 2017, American Chemical Society.

its performance with the gold standard ultracentrifugation techniques (**Fig. 4A**). This way, two nanoporous membranes were implemented on the disc. As the disc begun rotating with a low speed (<500g), the sample fluid reached the first nanofilter with a size of 600 nm. Particles larger than 600 nm in size remained behind the membrane, and smaller particles passed through it. Once again, smaller particles are faced with a second smaller nano filter with a pore size of 20 nm, allowing only smaller particles with a size smaller than 20 nm to move through. Therefore, particles with a size ranging between 20 nm and 600 nm (exosomes) were retained between two filters and collected for further analysis by the washing buffer injected through the microchannel. They also illustrated that the Exodisc device achieved 95% recovery of exosomes, and the entire procedure could be performed within 30 minutes.

Dehghani *et al.* utilized tangential flow filtration and normal flow filtration separation methods to isolate exosomes from purified human plasma using a PDMS microfluidic device (**Fig. 4B**) (Dehghani et al., 2019). A membrane with the pore size of 80 nm was employed to isolate exosomes based on their size. This paper reported that using normal flow membrane separation technology resulted in cake formation across the membrane, which reduced the capturing efficiency, increased the pressure drop, and finally, led to membrane clogging. On the other hand, the tangential flow method showed a high exosome isolation and recovery rate with a small amount of cake formation on the membrane surface. Moreover, they compared two types of membranes, including the conventional track-etch membrane and ultrathin silicon nitride nanomembranes in terms of pressure drop across the membranes and isolation efficiencies. They also compared the results derived from experiments with analytical solutions and COMSOL Multiphysics simulation results. Overall, the use of an ultrathin silicon nitride membrane showed a lower pressure drop so that the device was able to operate for a longer period of time. In addition, in ultrathin membranes, particles would not be trapped into the bulk of the membrane, and therefore, the exosome isolation and release efficiency would increase.

Chen et al. recently reported a label-free ultrafast-isolation system for exosome isolation in various biofluids such as saliva, plasma, tears, and culture medium (Chen et al., 2021). Briefly, the automation feature of this method enabled facile use and leveraged the reproducibility. Briefly, EXODUS is a dual filter sample reservoir with two outlets, each connected to a nanoporous anodic aluminum oxide (AAO) membrane. Periodic negative pressure oscillations are generated on the membranes by changing the negative pressure and air pressure direction. As the periodic negative pressure switches from one side of the device to the other, it not only allows small particles to pass through the membrane, but it also induces vibrations across the membrane to remove accumulated particles on the membrane and eliminate membrane fouling. As a result, the exosomes are gathered in the central chamber. Another advantage of this method can be listed as membranes of varying pore sizes are able to collect particles of different sizes with high purity, speed, and yield.

In another study, Wang *et al.* developed a microfluidic, silicon, nanowire-coated, ciliated micropillars device (**Fig. 4C**) (Wang et al., 2013). In this method, the micropillars array with 900 nm of interpillar spacing not only served as a support for nanowires, but also eliminated larger particles, such as cell debris and apoptotic bodies. Then, exosomes were trapped inside the nanowires depending on the interval between nanowires with a size range of 30-200 nm. The isolation experiment was completed within 10 minutes without the degradation of nanowires. To extract the trapped exosomes, the porous nanowires were dissolved overnight in the PBS buffer solution. The authors also reported that it was possible to increase the sensitivity and functionality of the device and explore the immunoaffinity-based isolation technique by using specific antibodies in porous silicon nanowires. The main shortcoming to these label-free approaches was the absence of specificity, and it was not possible to distinguish any particles with identical exosome sizes. Moreover, particle aggregation on the nanowires or nanoporous membrane could result in the membrane and microfluidic device being clogged, preventing further isolation of the target analytes.

Another size-based filtration technology that Liu *et al.* introduced is the Exosome Total Isolation Chip (ExoTIC) (Li et al., 2017a, 2017b), extracting exosomes from different clinical samples, including plasma, urine, and lung bronchoalveolar lavage, varying in size from 30 to 200 nm (Fig. 4D). In this platform, plastic housing with a ring-shaped gasket was used to prevent leakage from the device. In addition, the cellulose pad served as support to prevent the filter from deforming under pressure by inserting the syringe pump. Then, prefiltering the sample by a PES syringe filter, a constant flow rate syringe pump was used to introduce the sample into the system continuously. The exosome-enriched solution was then washed by PBS, and the purified exosome solution was gathered by a pipette and kept at $4^\circ C$ for further analysis. The results also pointed out that the exosome isolation yield using ExoTIC is 4-fold higher than the UC method. They also compared the ExoTIC device with commercially-available PEG precipitation kits, including ExoQuick and Macherey, and showed that the exosome isolation yield of ExoTIC was 3-4-fold higher. Eventually, membranes with different sizes, such as 30, 50, 80, 100, and 200 nm, were arranged in series to create a modular device to separate the exosome with different size ranges.

2.2.2. *Inertial lift force*

The inertial lift force is a passive approach used to isolate the exosomes in microfluidic devices. Under the inertial force, particles are located across the microchannels based on their difference in size and velocity between fluid and particles (Nieuwstadt et al., 2011). The researchers used a rapid inertial solution exchange (RInSE) technique (Gossett et al., 2012) *via* an inertial lifting force to sort the microparticles within the microchannel corresponding to their size (Dudani et al., 2015). Given the exosome nanoscale size and the fact that nanoparticles could not be affected by inertial forces, exosomes were first incubated with 20-micron polystyrene beads to create a large complex of exosome-beads. Inertial lift force pushes the beads into the microchannel center, where they were able to collect from the appropriate outlet and transfer the beads into the wash buffer solution. Since other non-exosomal EVs were not sufficiently large, they did not encounter inertial forces and stay within the channel walls, and did not travel toward the bead's outlet region. In this study, the bead isolation efficiency was reported as 100%. Once the beads were separated, they underwent centrifugation to remove the cell debris and undesired analytes to extract exosomes for further examination. While the fabricated microfluidic device had a high-throughput of 70 $\frac{\mu L}{min}$, which was five-fold higher than other microfluidic devices, it was not suitable for an emerging healthcare application for 4 hours of the incubation step (**Fig. 5A**).

In another study, Tay *et al.* utilized the inertial-based separation method in a curved channel, called the spiral inertial method, in order to isolate EVs from whole blood based on their size (Tay et al., 2017). In a curved channel, a secondary flow was generated due to induced rotational force. Dean flow, a rotational flow perpendicular to the main flow, could exert an additional drag force on the particle, and particles could be sorted based on their size (**Fig. 5B**). At the curvatures, an additional pressure gradient was induced to the flow, and consequently, the velocity profile and streamlines would be changed. Since the smaller particles experienced a lower lift force, they were positioned at the inner wall surface, and on the other hand, larger particles were located near the outer wall since they could tolerate a higher lift force. Compared with centrifugation methods, the morphology of the EVs was preserved properly.

2.2.3. *Viscoelastic flow*

Another size-based and label-free approach for exosome separation is viscoelastic microfluidic systems (Chiriacò et al., 2018; Li et al., 2017a, 2017b; Yuan et al., 2018). Since the elastic lifting force is proportional to the particle volume, it allows for the separation of the particles based on

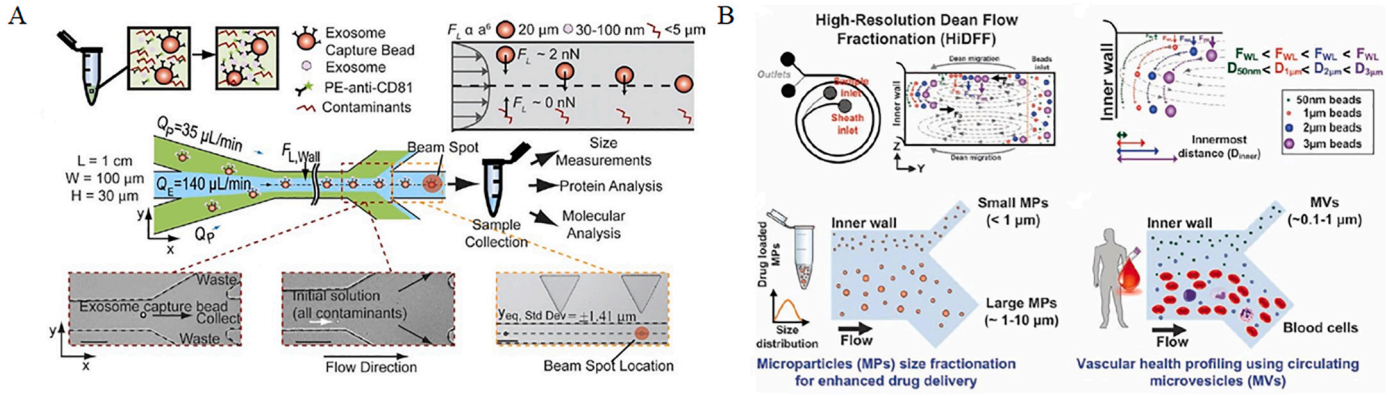

Fig. 5. Examples of microfluidic devices for exosome separation based on inertial lift force. (A) Exosomes are incubated with beads and then separated based on their size. Reused with permission from Ref. (Dudani et al., 2015). Copyright 2015, American Institute of Physics. (B) Spiral inertial microfluidic device, a combination of inertial force and dean flow fractionation for exosome isolation, is depicted. Reused with permission from Ref. (Tay et al., 2017). Copyright 2017, Nature Publishing Group.

their size. Liu *et al.*, for instance, utilized a high aspect ratio PDMS microfluidic device to separate exosomes from other larger EVs in cell culture media and fetal bovine serum (FBS) samples (Liu et al., 2017a, 2017b). A syringe pump was used to inject the sample fluid and sheath fluid containing a limited quantity of polyoxyethylene PEO (0.1 wt % PEO) into the microfluidic device from two inlet holes. The addition of PEO to the solution provided a viscoelastic environment that implements elastic forces to particles with different sizes, allowing the lateral position of nanoparticles to be regulated according to their size into the microchannels. Larger particles migrated more rapidly to the centerline and they were collected from the middle exit channel, while the smaller particles migrated more slowly and they were gathered from the two side outlets near the microchannel wall (**Fig. 6A**). This method resulted in high purity and >80% recovery rate. Compared with the inertial lift force method, this method did not require beads for size amplification since the elastic force was around one order of magnitude greater than the inertial lift force. In addition, compared with time-consuming ultracentrifugation method (several hours), the transportation time of exosomes in the microfluidic device was 0.1 second, minimizing any possible physical damages during the isolation process.

2.2.4. *Deterministic Lateral Displacement method*

The deterministic lateral displacement (DLD) method is a passive size-based method introduced (Huang et al., 2004). In this method, the microfluidic device consists of a periodic array of pillar obstacles with subsequent rows of the array displaced laterally by a certain distance, resulting in an array with an inclination with respect to the flow direction. The device operates in two distinct modes. Smaller particles are in zigzag mode and follow the streamlines (fluid flow direction), while the larger particles in displacement (bump) mode follow the array slope and are displaced laterally across an array. The cut-off diameter between zigzag mode and displacement mode is called the critical particle diameter, which can be determined by adjusting various device design parameters (Xavier et al., 2016). Huang *et al.* utilized this label-free continuous method to isolate particles with sizes of 0.8, 0.9, and 1.03 microns with a resolution of 10 nm (Huang et al., 2004). In another study, Santana *et al.* (Santana et al., 2014) implemented a DLD microfluidic device to isolate microvesicles from a heterogeneous group of EVs obtained from cancer cells. The particle recovery efficiency and purity were obtained as 39% and 98.5%, respectively. As another example, Wunsch *et al.* showed that a DLD method could separate particles in nanoscale sizes (nano-DLD), such as exosomes, using a nanofluidic silicon chip. Their results showed that at a low Peclet (Pe) number where the diffusion competes with deterministic displacement, particles in the size range of 20 nm to 110 nm could be separated with high resolution. Smith *et al.* utilized a nanoDLD chip with 1024 parallel arrays to isolate

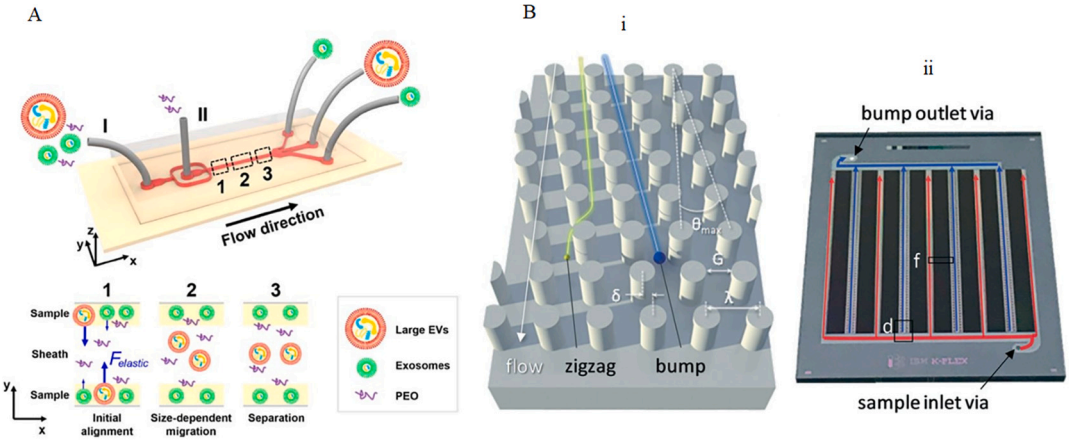

Fig. 6. Examples of microfluidic devices for exosome separation based on hydrodynamics of the system. (A) Viscoelastic exosome separation microfluidic device is presented. Sample and sheath fluid containing PEO are introduced to the microchannel. The particles are sorted and separated based on their size across the microchannel. Reused with permission from Ref. (Li et al., 2017a, 2017b). Copyright 2017, American Chemical Society. (B) Exosome separation is designed on nanoDLD pillar arrays (i), and the tilted-view image of the top side of nanoDLD chip (ii) is exhibited. Reused with permission from Ref. (Smith et al., 2018). Copyright 2018, Royal Society of Chemistry.

exosomes from urine and serum samples **(Fig. 6B)** (Smith et al., 2018). The device had high throughput with a flow rate of 900 μL/hr so that it holds the potential to be used in clinical applications for exosome isolation. Moreover, this device was also able to isolate exosomes with sizes ranging from 30-200 nm and provided 50% of recovery yield for serum and urine samples. Despite its high-resolution characteristics, other particles, including viruses and lipoproteins, can be co-isolated in this method. Clogging is another issue in DLD systems since the small particles can be trapped between the small gaps of the pillars. Moreover, not only is this method expensive, but also due to the large number of pillars in DLD based microfluidic devices, the fluid resistance is relatively high, which prevents large sample volume separation. There are some solutions to the mentioned limitations of this method, which has been addressed properly in the literature (Salafi et al., 2019).

2.2.5. *Acoustic waves*

Acoustic waves have elevated accuracy and biocompatibility in cells and bioparticle manipulation (Ding et al., 2014; Laurell et al., 2007; Li et al., 2015, 2016; Sharma et al., 2018a, 2018b; Taller et al., 2015; Wang et al., 2020). Acoustic nano filter is a contact-free and label-free promising exosome isolation technique, operating based on the differences in size. In this procedure, the sample is introduced into a chamber and exposed to ultrasound waves, where the radiation forces are applied to the particles, and the particles respond to the force based on their compressibility, size, and density (Ai et al., 2013; Bruus, 2012). Accordingly, particles have undergone acoustic radiation forces and move towards the pressure nodes. Stokes drag force counteracts the passage of particles to the pressure nodes. The acoustic force is proportional to volume of particles, while the drag force is proportional to the particle diameter. Therefore, for large particles, the acoustic radiation force is dominant over the drag force, and they experience a greater radiation force and move quickly to the pressure nodes, resulting in particle separation depending on the particle size. On the other hand, in tiny particles, the acoustic force and the drag force are comparable, so smaller particles have little lateral displacement. By varying the input power, it is possible to sort the particles proportional to their size and obtain the appropriate particle cut-off size. In a report, Lee *et al.* have stated that the separation efficiency using the acoustic nano filter system was more than 90%, and the cut-off size could be adjusted by varying the flow velocity and acoustic power so that particles with different sizes could be sorted out (Lee et al., 2015).

Wu *et al.* have employed an acoustofluidic device (the combination of microfluidics and acoustics) to separate an exosomes sample with a high purity and high yield from undiluted whole blood (**Fig. 7A**) (Wu et al., 2017). The constructed devices consisted of two sequential components based on surface acoustic wave formation. Cells and other debris were separated in the first part, and exosome isolation from other smaller EVs, such as apoptotic bodies and microvesicles, was the responsibility of the second part. The yield values of the first and second modules were 99% and 98.4%, respectively, along with >99.9% of total exosome isolation rate. The authors also claimed that their acoustofluidic device could separate exosomes from different samples, such as urine, blood, saliva, plasma, and breast milk. They shown that their device would have the potential to achieve a high isolation yield,

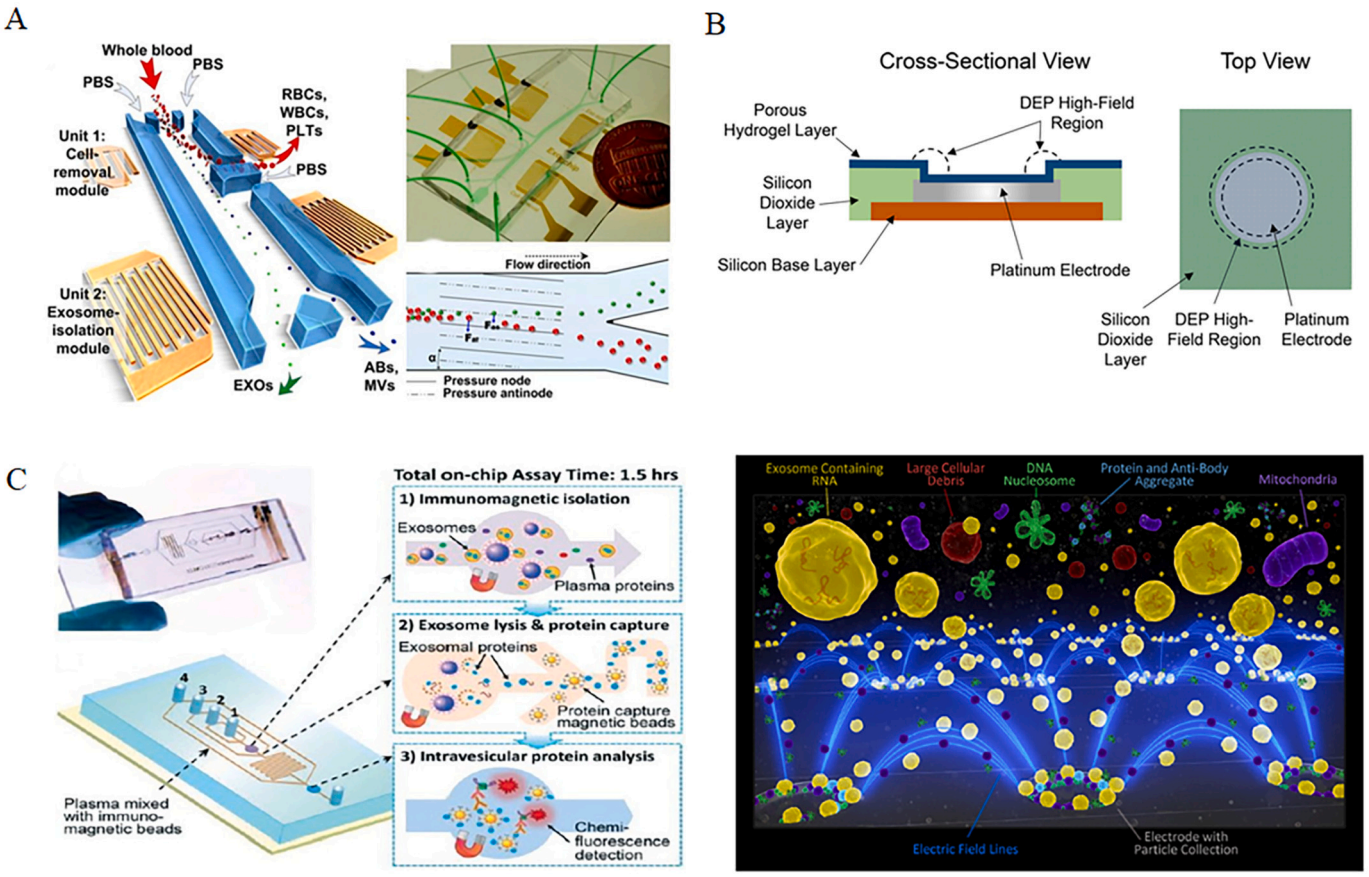

Fig. 7. The representative schematics for active microfluidic devices to separate exosomes through the implemented external force. (A) Acoustofluidic platform is used for exosome isolation from whole blood. The device consists of two modules so that larger particles are separated in the first module and the module is responsible for exosome isolation from smaller particles. Reused with permission from Ref. (Wu et al., 2017). Copyright 2017, National Academy of Sciences. (B) Dielectrophoretic-based microfluidic device is represented. Exosomes separation is applied through the electrophoretic force generated by an electrokinetic microarray. Reused with permission from Ref. (Ibsen et al., 2017). Copyright 2017, American Chemical Society. (C) Magnetic-based immunoaffinity microfluidic device is illustrated for exosome isolation from human plasma. Reused with permission from Ref. (He et al., 2014). Copyright 2014, Royal Society of Chemistry.

eliminate the time-consuming preprocessing of undiluted blood samples, and perform all separation steps in a single chip.

2.2.6. Dielectrophoretic and Electrophoretic techniques

When a particle is subjected to a non-uniform electrical field, it is polarized and experiences a force from the electrical field, and this phenomenon is called dielectrophoretic (DEP) (Esmaeilsabzali et al., 2013). The exerted dielectrophoretic force relies on the particle volume, absolute permittivity of the particle and solution, and the implemented electrical field intensity gradient value through the microchannel (Harrison et al., 2015; Mata-Gomez et al., 2016; Mohammadi et al., 2015; Shi et al., 2018). Typically, there are two different categories of DEP separation methods, including negative dielectrophoresis and positive dielectrophoresis. In positive dielectrophoresis, particles are more polarized compared with the surrounding solution and the particles move toward the high-intensity electrical field region. On the other hand, in negative dielectrophoresis, the solution is more polarized than the particles and the particles push to the lower intensity electrical field. Therefore, particles can be separated based on their size and dielectric properties (Gallo-Villanueva et al., 2011; Pysher and Hayes, 2007).

Although these methods are employed in some studies successfully (Ayala-Mar et al., 2019; Ibsen et al., 2017; Lewis et al., 2018; Shi et al., 2018), there is always the risk of localized heating due to the implemented voltage across the microfluidic device. Additionally, DEP-based techniques typically have fluid conductivity requirements that are incompatible with biofluids of physiological osmolarity. Ayala-Mar *et al.*, for instance, have utilized a PDMS microfluidic device to separate the exosomes from a pre-prepared sample using the dielectrophoretic method (Ayala-Mar et al., 2019). In this study, the authors used dielectrophoresis in two different sections to isolate two sub-populations of exosomes with different sizes. Exosomes were trapped by applying an electric potential difference of 2000V for 20 seconds. Once the exosomes were trapped, an electroosmotic flow was used to recover and release the exosomes without the need for a syringe pump. In another study, Ibsen *et al.* employed a microarray chip to isolate glioblastoma exosomes from undiluted human plasma samples (Ibsen et al., 2017). The isolation time was reported to be less than 15 min using a small volume of sample (30-50 μl). As reported, exosomes were attracted to the microelectrode edges, where the electrical field was high, while the larger particles moved toward the area between the electrodes where the dielectrophoretic field intensity was lower (**Fig. 7B**). The main limitation of this method was that the exosomes and the plasma sample would be in direct contact with the electrodes so that it would have an adverse effect on the bioparticles. In order to resolve this issue, microelectrodes were coated by a porous hydrogel layer. This layer not only protected exosomes by preventing their direct contact with the microelectrode, but also minimized bubble formation through electrolysis.

Compared with the DEP force, the electrophoretic force can be used to separate charged particles (Davies et al., 2012). As previously mentioned, considering that the exosomes are negatively charged particles, they can be isolated by an electrophoresis method. When a charged particle is exposed to an electrical field, two counteract forces affect the particles. The particle is accelerated due to the electrical field, and on the other hand, drag force hinders its speed. Therefore, after a while, the charged particle reaches its terminal velocity, where the electrical field and drag forces are balanced, which is a function of particle charge, size, and electrical field intensity. Specifically, Cho *et al.* have employed electrophoresis in conjugation of membrane filtration with a pore size of 30 nm to isolate EVs from mouse blood plasma (Cho et al., 2016). In this system, two membranes were used between two electrodes. When the flow passed through the channel, negatively charged particles migrated towards the anode, whereas neutral or positively charged particles moved towards the cathode electrode. As the particles reached the membrane surface, only particles smaller than 30 nm were able to pass through it, and in this way, the smaller particles could be isolated. This method resulted in a high yield and recovery rate, and compared with pressure-driven filtration, it eliminated the clogging problem, which typically occurred in passive filtration.

2.2.7. Affinity-based exosome isolation methods

Similar to conventional immunoaffinity-based exosome isolation methods, the same strategy can be implemented to microfluidic devices. This way, the exosomes are captured by the corresponding antibodies immobilized on the surface of the microfluidic chip, which target to exosome surface markers. The specificity of this method strongly depends on the affinity antibody. The immunoaffinity method in the microfluidic devices can be used either by immobilization of the specific antibodies on the microchannel surface or using magnetic beads (Meng et al., 2020). Chen *et al.*, for instance, have designed an immunoaffinity-based method on a microfluidic chip to separate exosomes from serum and blood samples without the need for subsequent ultracentrifugation of the sucrose DG (Chen et al., 2010). Their device included a herringbone structure on its ceiling to improve mixing and separation efficiency. Per their results, anti-CD63 antibodies coated microchannels could easily capture exosomes from 400 µl of cell culture samples. Moreover, by introducing a lysis buffer and air flushing to the captured exosomes on the microchannel surface, they were able to purify RNA. On the other hand, the direct RNA separation methods are basically hindered by the co-isolation of other biological components and contaminated with ipids and proteins. As shown in this study, the isolation of RNA from serum-derived exosomes on a microfluidic chip improves the extraction quality of RNA, providing a better alternative compared to the direct RNA extraction methods using blood or plasma. Around 30 ng of total RNA was obtained from the captured exosomes from a lung cancer patient. Compared with lengthy conventional isolation methods, the purification and extraction of exosomal RNA took an hour and only needed for 100-400 microliter of serum sample.

As it is evident, the greater the surface area of the microchannel, the more antibodies can be immobilized on the surface, leading to high recovery rate in exosome isolation. Considering this idea, Zhang *et al.* have used graphene oxide and polydopamine (GO/PDA) to provide a nanoporous structure and higher surface to immobilize more antibodies, and therefore, aimed to capture more exosomes (Zhang et al., 2016). In this study, anti-CD81 antibody could easily capture exosomes from human plasma. As a result, the embedding nanomaterials into the microfluidic device improved the exosome capture efficiency and prevented non-specific binding. In another study, Hisey *et al.* have employed herringbone grooves structure within the microfluidic device channel to increase the surface area, covalently functionalized with antibodies (Hisey et al., 2018). Tumor-derived exosomes were captured from the serum samples of ovarian cancer patients. Due to the novel microchannel structure designed in this study, the exosome separation yield was improved. Furthermore, the authors claimed that the antibody-antigen affinity bonding would be destroyed using glycine-HCl buffer at low pH levels, thus isolating intact and label-free exosomes-desirable for downstream analyses. Compared with magnetic bead-based immunoaffinity methods, the processing time was lower in this method; however, the reusability of the device should be carefully investigated since the buffer solution might damage the antibodies and affect their activity.

In another immunoaffinity-based method, He *et al.* utilized a magnetic bead-based method using a microfluidic device to capture the exosomes from human plasma (He et al., 2014). First, the sample was mixed with antibody-labeled magnetic beads and introduced to the device, where the magnetic beads were retained by an external magnetic source (**Fig. 7C**). Then, a lysis solution was added to the solution to release the intravesicular proteins of the captured exosomes. Once the intravesicular proteins were released, the antibody-labeled magnet beads were introduced from two sides of inlets to capture the proteins and retained in the second chamber by applying an external magnetic field. Afterwards, chemifluorescence reagents were added for sandwich immunodetection of desired proteins. Compared with conventional

methods, this method increased the sensitivity and reduced the required sample volume (30 μl) and processing time (100 min). Due to the characteristics of the microfluidic devices, high-throughput exosome isolation would be obtained by scaling up the device.

Despite its simplicity and low processing time, using the immunoaffinity-based method not only can isolate only specific exosomes that have target proteins on their surface (pan-exosome markers), but it also introduces impurities to the solution for downstream analysis along with labeling procedure. Moreover, due to the utilization of the antibodies in this process, this method is considered an expensive method. However, due to its high viscosity, presence of microvesicles of similar size, and non-specific proteins, the immunoaffinity method is the best choice in blood plasma samples.

3. Commercially available products

In this section, commercially available products for EVs are evaluated comprehensively (Table 3). At first glance, ExoMir (Bioo Scientific, Austin, TX, USA) is a commercial product that operates based on sequential ultrafiltration and separates bioparticles with respect to their size. In this method, a syringe composed of two membranes with 200 nm and 20 nm pore size is utilized. The sample is pre-treated through low-

Table 3
Commercially available products for exosome isolation.

Commercial products (Product and Company)	Advantages	Disadvantages	Sample	Sample Volume	Reference
Membrane-based Method					
ExoMir (Bioo Scientific, Austin, TX, USA)	• Cost-effective • Simple operation • Fast procedure • High separation efficiency	• Clogging	Serum	250 µl	(Andreu et al., 2016)
Precipitation-based Methods					
EXO-Prep (HasnaBioMed Life Sciences, Estonia)	• High-throughput • Simple operation • High yield	• Low purity due to the contamination with co-precipitated reagents • Pre and post cleanup steps required • Time-consuming • Contaminants affect downstream analysis	Plasma	1.5 mL	(Slyusarenko et al., 2021b)
Exosome Purification Kit (Norgen Biotek, Canada)			Serum, Plasma	1 mL	(Martínez-González et al., 2020)
Exo-spin Isolation Kit (Cell Guidance Systems, USA)			Cell Culture	1 mL	(Li et al., 2020a, 2020b)
ExoQuick Exosome Precipitation (System Biosciences, CA, USA)			Serum, Plasma	100-250 µl	(Hannafon et al., 2016; Tang et al., 2017; Wang et al., 2017)
PureExo Exosome Isolation Kit (101 Bio, Palo Alto, CA)			Plasma	10 mL	(Wang et al., 2018)
miRCURY Exosome Isolation Kit (Exiqon, Denmark)			Serum, Plasma	1 mL	(Martínez-González et al., 2020)
Total Exosome Isolation Reagent (Invitrogen, Carlsbad, CA, USA)			Serum, Plasma	1-1.5 mL	(Martínez-González et al., 2020; Slyusarenko et al., 2021b)
Minute High-Efficiency Exosome Precipitation Reagent (Invent Biotechnologies, USA)			Serum	100 µl	(Gao et al., 2019)
RIBO Exosome Isolation Reagent (RIBO, Guangzhou, China)			Plasma, Cell culture	3-5 mL	(Sun et al., 2020)
Size-exclusion chromatography					
qEV (iZON Science)	• High purity • Intact stucture of isolated exosomes • Capable of processing various types of samples • Good reproducibility • Needing for low sample volume • Exosome preservation	• Time-consuming • Low recovery • Relatively expensive and complex device • Low-throughput	Serum	4 mL	(Smith et al., 2018)
EVSecond (GL Sciences)			Cerebrospinal fluid	1 mL	(Sjoqvist et al., 2020)
ExoLutE (Rosetta Exosome Company, Korea)			Serum	500 µl	(Kim et al., 2020)
PURE-Evs (HansaBioMed)			Saliva	250 µl	(Han et al., 2020)
Immunoaffinity-based Methods					
Exosome-Human EpCAM isolation reagent (Thermofisher)	• Simple operation • High purity and specificity	• Challenges related to finding appropriate antibody • Expensive • Antibody masking with interfering particles • Antibody cross-reactivity • Low yield • Low-throughput	Cell Culture	100 µl	(Hannafon et al., 2016)
Exosome Isolation Kit CD81/CD63 (Miltenyi Biotec)			Cell culture supernatant	100-300 µl	(Koliha et al., 2016)
Exosome Isolation and Analysis kit (Abcam)			Serum	100 µl	(Gao et al., 2019)
MagCapture Exosome Isolation Kit PS (FUJIFILM Wako Pure Chemical Corporation, Japan)			Serum Urine	50 µl 500 µl	(Nakai et al., 2016)
Membrane Affinity Spin Column Method					
ExoEasy Maxi Kit (Qiagen)	• Simple operation • High-throughput	• Low purity	Serum, Plasma	0.2-4 ml	(Smith et al., 2018; Zheng et al., 2018)
Capturem Exosome Isolation Kit (Takara Bio, Europe)	• Rapid isolation		Cell culture supernatant	-	(Srivastava et al., 2020)
Microfluidic Immunoaffinity-based Methods					
ExoChip	• High-throughput • Simple operation	• Antibody stability • Challenges related to finding appropriate antibody	Serum	400 µl	(Kanwar et al., 2014)
ExoSearch			Plasma	20 µl	(Zhao et al., 2016)

speed centrifugation to separate larger particles and cell debris, and also, to prevent clogging the membrane. Then, the sample passes through the 200 nm membrane, and the passed sample again travels through the second membrane with the pore size of 20 nm. The desired product is trapped between two membranes and can be used for further analysis (Doyle and Wang, 2019). This method is simple, rapid, and easy-to-operate. However, membrane clogging is possible, and exosomes would be damaged due to shear stress and centrifugation.

Precipitation-based commercial kits include EXO-Prep (HasnaBioMed Life Sciences, Estonia) (Slyusarenko et al., 2021a), Exosome Purification Kit (Norgen Biotek, Canada) (Martínez-González et al., 2020), Exo-spin Isolation Kit (Cell Guidance Systems, USA), which combines precipitation and size-exclusion chromatography techniques (Li et al., 2020a, 2020b), ExoQuick exosome precipitation (System Biosciences, CA, USA) (Wu et al., 2021), PureExo Exosome Isolation Kit (101 Bio, Palo Alto, CA) (Wang et al., 2018), miRCURY exosome isolation kit (Exiqon, Denmark) (Danarto et al., 2020), Total Exosome Isolation Reagent (Invitrogen, Carlsbad, CA, USA) (Imai et al., 2021), Minute High-Efficiency Exosome Precipitation Reagent (Invent Biotechnologies, USA) (Gao et al., 2019), and RIBO Exosome Isolation Reagent (a precipitation reagent for plasma or serum) (RIBO, Guangzhou, China) (Sun et al., 2020). These methods utilize various reagents, such as polymeric substances, to precipitate exosomes in a solution through a low centrifugation speed (10000-20000 ×g) (Lane et al., 2015). Researchers have compared various commercial kits and conventional methods, and their results state that the commercial products demonstrate lower user dependency, lower processing time, high purity, and high yield (Alvarez et al., 2012; Ding et al., 2018; Helwa et al., 2017; Paolini et al., 2016; Patel et al., 2019; Skottvoll et al., 2018). Despite their high efficiency and throughputness, the main limitation of these methods is that introducing reagents into the solution results in considerable impurities for downstream analysis. Therefore, the purity is relatively low, and pre- and post-cleaning processes are required.

The qEV column (iZON Science) (Smith et al., 2018), EVSecond (GL Sciences) (Sjoqvist et al., 2020), ExoLutE (Rosetta Exosome Company, Korea) (Kim et al., 2020), and PURE-Evs (HansaBioMed) (Han et al., 2020), are size-exclusion chromatography commercial products. In these methods, the sample first experiences low-speed centrifugation for a few minutes (usually 20 min) to separate larger cell debris and proteins. Then, the pretreated sample is introduced to the size-exclusion column followed by elution with PBS. Afterward, the required eluted fraction of the sample is collected and concentrated for further analysis. As an example, Lobb *et al.* have showed that for isolation of particles smaller than 100 nm, ExoQuick and Exo-spin products provided higher yield than qEV columns and OptiPrep DG methods (Lobb et al., 2015). Using human plasma, they also showed that although the qEV column had the lowest exosome recovery rate, it isolates exosomes with the highest purity.

The Exosome-Human EpCAM isolation reagent (Thermo Fisher) is an immunoaffinity-based commercially available product (Ji et al., 2021). Using this method, EpCAM markers on exosomes can be identified to diagnose the EpCAM-related cancers. In this method, Dynabeads magnetic beads are used to selectively capture the exosome through magnetic separation technology. The Exosome Isolation Kit CD81/CD63 (Miltenyi Biotec) (Koliha et al., 2016), and Exosome Isolation and Analysis kit (Abcam) (Gao et al., 2019) are similar to the Exosome-Human EpCAM kit, although the antibody reagent is different in these methods. The MagCapture Exosome Isolation Kit PS (FUJIFILM Wako Pure Chemical Corporation, Japan) is another immunoaffinity commercial product that utilizes magnetic beads and phosphatidylserine (PS)-binding protein for isolating exosomes from culture medium and bodily fluids (Nakai et al., 2016).

The ExoEasy Maxi Kit (Qiagen) is a membrane affinity spin column method for exosome purification. Here, exosomes can be isolated from various samples, including plasma, serum, and cell culture supernatant, and the process takes only 25 min. Briefly, the pretreated sample is first mixed with a buffer XPB solution bound to the ExoEasy membrane affinity spin column. Then, the exosomes are washed with a buffer and eluted with buffer XE solution. This method is based on the generic and biochemical characteristics of vesicles to recover the full spectrum of EVs (Buschmann et al., 2018; Del Re et al., 2019). The Capturem exosome isolation kit (Takara Bio, Europe) (Srivastava et al., 2020) is another membrane affinity spin column method for exosome isolation, and it isolates exosomes from cell culture supernatant media within 30 min. In this method, the sample is introduced to the column, and then, experienced low speed centrifugation for few minutes. Attached exosomes to the column are washed and eventually isolated using a buffer solution.

ExoChip is one of the commercially available immunoaffinity-based microfluidic devices for exosome isolation (Kanwar et al., 2014). In this product, anti-CD63 antibody is immobilized on the surface of the microchannels to capture exosomes since CD63 proteins are exosomal markers existing in various cells. Specifically, in this study, exosomes isolated from serum samples of healthy and pancreatic cancer patients were analyzed. The microfluidic device was designed so that the fluid velocity was different in various sections of the device so that the exosomes had enough retention time to interact with immobilized antibodies. In this method, a fluorescent dye was used for exosome quantification so that extravascular vesicles could be visualized and measured through a plate reader under the microscope. ExoSearch is another microfluidic device for exosome isolation from blood plasma using immunomagnetic beads that utilizes 20 μl of samples for the isolation and detection of three tumor proteins within 40 mins (Zhao et al., 2016).

4. Comparison and challenges of exosome isolation techniques

As explicitly stated in this review, exosomes can be used as surrogate biomarkers for disease diagnosis and therapeutic applications; thereby, it is vital to separate them from complex biospecimens for further analysis. At the end of any procedure, it is desired to obtain an intact homogenous exosome with uniform size distribution with high-yield and recovery. All the methods aforementioned pose their advantages and drawbacks, which should be considered before selecting the appropriate method. In this section, the characteristics and features of each method are briefly discussed.

The ultracentrifugation technique—a gold standard and well-established method for extracellular isolation, can be readily used in conjugation with other isolation methods to achieve high separation resolutions and yields. Still, the isolation yield enormously depends on the user and the apparatus used. Moreover, centrifugation techniques require not only expensive instrumentation, but also time-consuming procedures. The main advantage of the DG UC approach is that different layers with distinct densities can be generated, so that particles can be located according to their buoyant density in different layers. Therefore, it would be easy to distinguish between different types of EVs in this way. However, the DG UC method takes more time for sample purification compared to the ultracentrifugation methods.

Despite its rapid turnaround, high facility, and high yield, the precipitation-based isolation method needs further purification steps through removing precipitating reagents for downstream analysis. Immunoaffinity methods operate through physical and chemical characteristics of exosomes, relying on the interaction between antibodies and surface antigens of exosomes. The main advantage of this method is high specificity and high-purity in the exosome isolation. It is also easy to implement this idea in microfluidic devices by immobilizing specific antibodies to capture exosomes in the microchannels. However, this method requires a critical know-how on exosome biogenesis and biomarker information, thereby finding appropriate antibodies for specific applications. Despite all of these benefits mentioned, this method is interfered by the functionality loss of exosomes after detachment from the antibodies, and also, the instability of antibodies for long-term storage.

Size-exclusion chromatography has a short processing time since the separation is conducted in a single step. Considering the fact that no extra reagent is added, the separation efficiency is high, and high purified exosomes can be obtained. The main drawback of this method is that only a small amount of sample can proceed and a large amount of sample hampers the separation efficiency. Moreover, the possibility of co-precipitation of non-exosomal small particles should be carefully considered. Filtration—another facile exosome isolation strategy—works properly with microfluidic devices, and it also allows a high volume of samples being processed within a short period of time. As this method does not require the addition of extra reagents, a high purity of exosomes could be acquired. Nevertheless, as a label-free method, this method lacks specificity so other particles with the same size could be isolated. Apart from these points, the possibility of the membrane clogging cannot be ignored as exosomes and other EVs could be trapped into the membrane pores, and eventually, they cause clogging. Similar to all size-based, label-free separation methods, inertial lift, viscoelastic, DLD methods cannot provide a high specificity. In particular, considering that exosomes are affected by inertial lift force due to their nanoscale size, they should be incubated with micron-sized beads to form exosome-bead conjugates. The main pros of the viscoelastic method are that there is no need for the use of beads, as the viscoelastic force is higher than the inertial force and can affect the exosomes. In addition, the time required for isolation using microfluidic devices is much lower than conventional size-based methods. Although the DLD method exhibits a high resolution, it has several obstacles, including the paucity of specificity, high-cost, clogging of the array, high pressure drops, resistance against fluid flow, and high shear stress exerted on the exosomes. Compared with passive methods, active techniques, including acoustic waves and electrophoretic and magnetic immunoaffinity methods, have a higher isolation throughput and efficiency, although external force is required to actuate the system; thus, the device complexity increases. Furthermore, the electrophoretic method is limited to the applications for a variety of biofluids due to the generated local heating induced by a high voltage. Among active methods, the magnetic immunoaffinity method holds the highest specificity due to the specific interaction of specific antibodies and exosomes.

5. Future outlook and conclusion

Exosomes hold great potential to be biomarkers that can be used in clinical applications for disease detection and therapeutics. Therefore, finding an efficient way for exosome separation is always required. Conventional isolation techniques however suffer from handling and application limitations, such as low processing sample volume, sample pre- and post-processing, and structural damages on exosomes during the isolation process due to the exerted forces. While commercially available exosome isolation kits have several advantages, including time efficiency, high-yield, and ease-of-use, they are expensive, have low purity, and cannot be used to isolate exosomes from a wide range of complex bodily fluids. On the other hand, microfluidic-based methods are emerging methods in exosome separation, and they are promising alternatives to gold standard conventional methods, thereby addressing their existing limitations. Moreover, microfluidic devices have several advantages, including low sample consumption, miniaturized size, low-cost, rapid turnaround, high recovery, and high sensitivity, making them suitable for clinical application, especially for the realm of personalized medicine. Based on their physical, chemical, and biological properties, exosomes can be separated using microfluidic devices.

In this review, we have presented a variety of microfluidic-based exosome isolation techniques. Today, microfluidic-based isolation methods focus on exosome separation corresponding to their physical properties, such as density and size, or biomarker properties through antibody and antigen interactions. The immuno-affinity process has piqued the interest of researchers due to its specificity, facile use, and application. However, not only is finding specific antibodies for each specific analyte challenging, but the stability of the antibodies and reusability of the device should be thoroughly investigated. Considering that this approach can adversely affect the biological properties of exosomes, there is a need for label-free, microfluidic-based exosome isolation techniques. Furthermore, several size-based microfluidic chips are introduced as proficient methods for exosome isolation, which do not require any external forces and antibodies, although they suffer from clogging and size overlapping problems. However, neither the immunoaffinity nor the size-based methods can effectively distinguish exosomes from other nano-sized particles in the sample. Therefore, combining these two methods could open up a new avenue for investigating a desirable approach to improve isolation purity, hence making easier to analyze exosomes in clinical research.

While numerous microfluidic-based isolation methods are introduced for this manner, implementing these devices on an industrial scale is also challenging. To process a large volume of samples, it is necessary to construct new devices to achieve high exosome purity and high-throughput. In particular, as compared to conventional photolithography methods, 3D printing methods have provided better physical and chemical properties, a lower cost, and a high throughput prototype, making it ideal for industrial applications (Akceoglu et al., 2021). Besides, sample collection and pre- and post-treatment are the crucial steps that a trained person should take in consideration. To adapt a microfluidic device to clinical use, the complexity issue must be reduced, and it has to be fully automated so that it can be operated by an untrained personnel. User-friendly microfluidic devices would accelerate their utility and applicability in clinical purposes.

Currently, microfluidic devices are utilized for bulk exosome isolation and analysis. To have a better insight into exosome function, shape, and size, it would be better to design a highly sensitive and high-throughput microfluidic device to analyze single exosomes with higher accuracy. In the future, fabricating single exosome isolation and analysis-based microfluidic chips would be a powerful tool for cancer diagnosis. In conclusion, it is worth mentioning that microfluidic devices have been successfully implemented for exosome isolation from different samples, including human blood plasma, serum, and urine. Each microfluidic-based method has some benefits and drawbacks, and there is no single device that can be used for all types of samples efficiently. Moreover, microfluidics would be more versatile when they are integrated with a sensor unit (Ahmed et al., 2020; Saylan and Denizli, 2018), and therefore, they not only enable the isolation of exosomes, but also allow their on-site analysis on a sensor (Mataji-Kojouri et al., 2020).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Dr. Fatih Inci (F.I.) gratefully acknowledges the support from TÜBİTAK 2232-International Fellowship for Outstanding Researchers (Project No: 118C254), TÜBİTAK 1001-The Scientific and Technological Research Projects Funding Program (Project No: 120Z445), and TÜBİTAK 3501-Career Development Program (CAREER) (Project No: 120Z335). This publication was produced benefiting from the 2232-International Fellowship for Outstanding Researchers Program of TÜBİTAK (Project No: 118C254). However, the entire responsibility of the publication/paper belongs to the owner of the publication/paper. The financial support received from TÜBİTAK does not mean that the content of the publication is approved in a scientific sense by TÜBİTAK. This work was supported by the BAGEP Award of the Science Academy. The authors also would like to thank Mr. Mehmet Tascioglu for carefully editing the manuscript.

References

Admyre, C., Johansson, S.M., Qazi, K.R., Filén, J.-J., Lahesmaa, R., Norman, M., Neve, E. P.A., Scheynius, A., Gabrielsson, S., 2007. Exosomes with Immune Modulatory Features Are Present in Human Breast Milk. J. Immunol. 179, 1969–1978. https://doi.org/10.4049/jimmunol.179.3.1969.

Ahmed, R., Ozen, M.O., Karaaslan, M.G., Prator, C.A., Thanh, C., Kumar, S., Torres, L., Iyer, N., Munter, S., Southern, S., Henrich, T.J., Inci, F., Demirci, U., 2020. Tunable Fano-Resonant Metasurfaces on a Disposable Plastic-Template for Multimodal and Multiplex Biosensing. Adv. Mater. https://doi.org/10.1002/adma.201907160.

Ai, Y., Sanders, C.K., Marrone, B.L., 2013. Separation of Escherichia coli bacteria from peripheral blood mononuclear cells using standing surface acoustic waves. Anal. Chem. 85, 9126–9134.

Akceoglu, G.A., Saylan, Y., Inci, F., 2021. A Snapshot of Microfluidics in Point-of-Care. Multifaceted Integrity with Materials and Sensors. Adv. Mater. Technol, Diagnostics. https://doi.org/10.1002/admt.202100049.

Al-Nedawi, K., Meehan, B., Micallef, J., Lhotak, V., May, L., Guha, A., Rak, J., 2008. Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat. Cell Biol. 10, 619–624.

Alvarez, M.L., Khosroheidari, M., Kanchi Ravi, R., Distefano, J.K., 2012. Comparison of protein, microRNA, and mRNA yields using different methods of urinary exosome isolation for the discovery of kidney disease biomarkers. Kidney Int. 82, 1024–1032. https://doi.org/10.1038/ki.2012.256.

An, M., Lohse, I., Tan, Z., Zhu, J., Wu, J., Kurapati, H., Morgan, M.A., Lawrence, T.S., Cuneo, K.C., Lubman, D.M., 2017. Quantitative proteomic analysis of serum exosomes from patients with locally advanced pancreatic cancer undergoing chemoradiotherapy. J. Proteome Res. 16, 1763–1772.

Andreu, Z., Rivas, E., Sanguino-Pascual, A., Lamana, A., Marazuela, M., González-Alvaro, I., Sánchez-Madrid, F., de la Fuente, H., Yáñez-Mó, M., 2016. Comparative analysis of EV isolation procedures for miRNAs detection in serum samples. J. Extracell. Vesicles 5. https://doi.org/10.3402/jev.v5.31655.

Asghar, W., Shafiee, H., Velasco, V., Sah, V.R., Guo, S., El Assal, R., Inci, F., Rajagopalan, A., Jahangir, M., Anchan, R.M., Mutter, G.L., Ozkan, M., Ozkan, C.S., Demirci, U., 2016. Toxicology Study of Single-walled Carbon Nanotubes and Reduced Graphene Oxide in Human Sperm. Sci. Rep. https://doi.org/10.1038/srep30270.

Ayala-Mar, S., Perez-Gonzalez, V.H., Mata-Gómez, M.A., Gallo-Villanueva, R.C., González-Valdez, J., 2019. Electrokinetically Driven Exosome Separation and Concentration Using Dielectrophoretic-Enhanced PDMS-Based Microfluidics. Anal. Chem. 91, 14975–14982. https://doi.org/10.1021/acs.analchem.9b03448.

Ayoib, A., Hashim, U., Gopinath, S.C.B., Thivina, V., Arshad, M.K.M., 2020. Design and fabrication of PDMS microfluidics device for rapid and label-free DNA detection. Appl. Phys. A Mater. Sci. Process. 126, 1–8. https://doi.org/10.1007/s00339-020-3337-7.

Ban, J.-J., Lee, M., Im, W., Kim, M., 2015. Low pH increases the yield of exosome isolation. Biochem. Biophys. Res. Commun. 461, 76–79.

Baranyai, T., Herczeg, K., Onódi, Z., Voszka, I., Módos, K., Marton, N., Nagy, G., Mäger, I., Wood, M.J., El Andaloussi, S., 2015. Isolation of exosomes from blood plasma: qualitative and quantitative comparison of ultracentrifugation and size exclusion chromatography methods. PLoS One 10, e0145686.

Barile, L., Vassalli, G., 2017. Exosomes: Therapy delivery tools and biomarkers of diseases. Pharmacol. Ther. 174, 63–78.

Böing, A.N., Van Der Pol, E., Grootemaat, A.E., Coumans, F.A.W., Sturk, A., Nieuwland, R., 2014. Single-step isolation of extracellular vesicles by size-exclusion chromatography. J. Extracell. vesicles 3, 23430.

Brennan, K., Martin, K., FitzGerald, S.P., O'Sullivan, J., Wu, Y., Blanco, A., Richardson, C., Mc Gee, M.M., 2020. A comparison of methods for the isolation and separation of extracellular vesicles from protein and lipid particles in human serum. Sci. Rep. 10, 1–13.

Brown, P.N., Yin, H., 2017. Polymer-based purification of extracellular vesicles. In: Extracellular Vesicles. Springer, pp. 91–103.

Brownlee, Z., Lynn, K.D., Thorpe, P.E., Schroit, A.J., 2014. A novel "salting-out" procedure for the isolation of tumor-derived exosomes. J. Immunol. Methods 407, 120–126.

Bruus, H., 2012. Acoustofluidics 7: The acoustic radiation force on small particles. Lab Chip 12, 1014–1021.

Busatto, S., Vilanilam, G., Ticer, T., Lin, W.-L., Dickson, D., Shapiro, S., Bergese, P., Wolfram, J., 2018. Tangential Flow Filtration for Highly Efficient Concentration of Extracellular Vesicles from Large Volumes of Fluid. Cells 7, 273. https://doi.org/10.3390/cells7120273.

Buschmann, D., Kirchner, B., Hermann, S., Märte, M., Wurmser, C., Brandes, F., Kotschote, S., Bonin, M., Steinlein, O.K., Pfaffl, M.W., Schelling, G., Reithmair, M., 2018. Evaluation of serum extracellular vesicle isolation methods for profiling miRNAs by next-generation sequencing. J. Extracell. Vesicles 7. https://doi.org/10.1080/20013078.2018.1481321.

Cantin, R., Diou, J., Bélanger, D., Tremblay, A.M., Gilbert, C., 2008. Discrimination between exosomes and HIV-1: Purification of both vesicles from cell-free supernatants. J. Immunol. Methods 338, 21–30. https://doi.org/10.1016/j.jim.2008.07.007.

Chen, C., Skog, J., Hsu, C.-H., Lessard, R.T., Balaj, L., Wurdinger, T., Carter, B.S., Breakefield, X.O., Toner, M., Irimia, D., 2010. Microfluidic isolation and transcriptome analysis of serum microvesicles. Lab Chip 10, 505–511.

Chen, H., Zeng, Y., Liu, W., Zhao, S., Wu, J., Du, Y., 2013. Multifaceted applications of nanomaterials in cell engineering and therapy. Biotechnol. Adv. 31, 638–653. https://doi.org/10.1016/j.biotechadv.2012.08.002.

Chen, G., Huang, A.C., Zhang, W., Zhang, G., Wu, M., Xu, W., Yu, Z., Yang, J., Wang, B., Sun, H., 2018. Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 560, 382–386.

Chen, Y., Zhu, Q., Cheng, L., Wang, Y., Li, M., Yang, Q., Hu, L., Lou, D., Li, J., Dong, X., Lee, L.P., Liu, F., 2021. Exosome detection via the ultrafast-isolation system: EXODUS. Nat. Methods 18, 212–218. https://doi.org/10.1038/s41592-020-01034-x.

Cheruvanky, A., Zhou, H., Pisitkun, T., Kopp, J.B., Knepper, M.A., Yuen, P.S.T., Star, R. A., 2007. Rapid isolation of urinary exosomal biomarkers using a nanomembrane ultrafiltration concentrator. Am. J. Physiol. Ren. Physiol. https://doi.org/10.1152/ajprenal.00434.2006.

Chiou, N.-T., Ansel, K.M., 2016. Improved exosome isolation by sucrose gradient fractionation of ultracentrifuged crude exosome pellets. Protoc. Exch. 10.

Chiriacò, M.S., Bianco, M., Nigro, A., Primiceri, E., Ferrara, F., Romano, A., Quattrini, A., Furlan, R., Arima, V., Maruccio, G., 2018. Lab-on-chip for exosomes and microvesicles detection and characterization. Sensors 18, 3175.

Cho, S., Jo, W., Heo, Y., Kang, J.Y., Kwak, R., Park, J., 2016. Isolation of extracellular vesicle from blood plasma using electrophoretic migration through porous membrane. Sensors Actuators B Chem. 233, 289–297. https://doi.org/10.1016/j.snb.2016.04.091.

Chung, K.Y., Quek, J.M., Neo, S.H., Too, H.P., 2020. Polymer-Based Precipitation of Extracellular Vesicular miRNAs from Serum Improve Gastric Cancer miRNA Biomarker Performance. J. Mol. Diagnostics 22, 610–618. https://doi.org/10.1016/j.jmoldx.2020.01.016.

Clayton, A., Court, J., Navabi, H., Adams, M., Mason, M.D., Hobot, J.A., Newman, G.R., Jasani, B., 2001. Analysis of antigen presenting cell derived exosomes, based on immuno-magnetic isolation and flow cytometry. J. Immunol. Methods 247, 163–174.

Colombo, M., Moita, C., van Niel, G., Kowal, J., Vigneron, J., Benaroch, P., Manel, N., Moita, L.F., Théry, C., Raposo, G., 2013. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J. Cell Sci. 126, 5553–5565.

Cooper, T.T., Sherman, S.E., Dayarathna, T., Bell, G.I., Ma, J., McRae, D.M., Lagugné-Labarthet, F., Pasternak, S.H., Lajoie, G.A., Hess, D.A., 2020. Ultrafiltration segregates tissue regenerative stimuli harboured within and independent of extracellular vesicles. bioRxiv. https://doi.org/10.1101/2020.01.28.923037.

Cutler, P., 2004. Immunoaffinity chromatography. In: Protein Purification Protocols. Springer, pp. 167–177.

Danarto, R., Astuti, I., Umbas, R., Haryana, S.M., 2020. Urine miR-21-5p and miR-200c-3p as potential non-invasive biomarkers in patients with prostate cancer. Turkish J. Urol. 46, 26–30. https://doi.org/10.5152/tud.2019.19163.

Davies, R.T., Kim, J., Jang, S.C., Choi, E.J., Gho, Y.S., Park, J., 2012. Microfluidic filtration system to isolate extracellular vesicles from blood. Lab Chip. https://doi.org/10.1039/c2lc41006k.

Dehghani, M., Lucas, K., Flax, J., McGrath, J., Gaborski, T., 2019. Tangential flow microfluidics for the capture and release of nanoparticles and extracellular vesicles on conventional and ultrathin membranes. Adv. Mater. Technol. 4, 1900539.

del Conde, I., Shrimpton, C.N., Thiagarajan, P., López, J.A., 2005. Tissue-factor–bearing microvesicles arise from lipid rafts and fuse with activated platelets to initiate coagulation. Blood 106, 1604–1611.

Del Re, M., Bertolini, I., Crucitta, S., Fontanelli, L., Rofi, E., De Angelis, C., Diodati, L., Cavallero, D., Gianfilippo, G., Salvadori, B., Fogli, S., Falcone, A., Scatena, C., Naccarato, A.G., Roncella, M., Ghilli, M., Morganti, R., Fontana, A., Danesi, R., 2019. Overexpression of TK1 and CDK9 in plasma-derived exosomes is associated with clinical resistance to CDK4/6 inhibitors in metastatic breast cancer patients. Breast Cancer Res. Treat. 178, 57–62. https://doi.org/10.1007/s10549-019-05365-y.

Deregibus, M.C., Figliolini, F., D'antico, S., Manzini, P.M., Pasquino, C., De Lena, M., Tetta, C., Brizzi, M.F., Camussi, G., 2016. Charge-based precipitation of extracellular vesicles. Int. J. Mol. Med. 38, 1359–1366.

Deshmukh, S., Inci, F., Karaaslan, M.G., Ogut, M.G., Duncan, D., Klevan, L., Duncan, G., Demirci, U., 2020. A confirmatory test for sperm in sexual assault samples using a microfluidic-integrated cell phone imaging system. Forensic Sci. Int. Genet. https://doi.org/10.1016/j.fsigen.2020.102313.

Dettenhofer, M., Yu, X.-F., 1999. Highly purified human immunodeficiency virus type 1 reveals a virtual absence of Vif in virions. J. Virol. 73, 1460–1467.

Ding, X., Peng, Z., Lin, S.-C.S., Geri, M., Li, S., Li, P., Chen, Y., Dao, M., Suresh, S., Huang, T.J., 2014. Cell separation using tilted-angle standing surface acoustic waves. Proc. Natl. Acad. Sci. 111, 12992–12997.

Ding, M., Wang, C., Lu, X., Zhang, Cuiping, Zhou, Z., Chen, X., Zhang, C.Y., Zen, K., Zhang, Chunni, 2018. Comparison of commercial exosome isolation kits for circulating exosomal microRNA profiling. Anal. Bioanal. Chem. 410, 3805–3814. https://doi.org/10.1007/s00216-018-1052-4.

Dong, L., Lin, W., Qi, P., Xu, M., Wu, X., Ni, S., Huang, D., Weng, W., Tan, C., Sheng, W., 2016. Circulating long RNAs in serum extracellular vesicles: their characterization and potential application as biomarkers for diagnosis of colorectal cancer. Cancer Epidemiol. Prev. Biomarkers 25, 1158–1166.

Doyle, L., Wang, M., 2019. Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells 8, 727. https://doi.org/10.3390/cells8070727.

Dudani, J.S., Gossett, D.R., Tse, H.T.K., Lamm, R.J., Kulkarni, R.P., Di Carlo, D., 2015. Rapid inertial solution exchange for enrichment and flow cytometric detection of microvesicles. Biomicrofluidics 9, 14112.

Enderle, D., Spiel, A., Coticchia, C.M., Berghoff, E., Mueller, R., Schlumpberger, M., Sprenger-Haussels, M., Shaffer, J.M., Lader, E., Skog, J., 2015. Characterization of RNA from exosomes and other extracellular vesicles isolated by a novel spin column-based method. PLoS One 10, e0136133.

Esmaeilsabzali, H., Beischlag, T.V., Cox, M.E., Parameswaran, A.M., Park, E.J., 2013. Detection and isolation of circulating tumor cells: Principles and methods. Biotechnol. Adv. 31, 1063–1084. https://doi.org/10.1016/j.biotechadv.2013.08.016.

Farooqi, A.A., Desai, N.N., Qureshi, M.Z., Librelotto, D.R.N., Gasparri, M.L., Bishayee, A., Nabavi, S.M., Curti, V., Daglia, M., 2018. Exosome biogenesis, bioactivities and functions as new delivery systems of natural compounds. Biotechnol. Adv. 36, 328–334. https://doi.org/10.1016/j.biotechadv.2017.12.010.

Fekete, S., Beck, A., Veuthey, J.-L., Guillarme, D., 2014. Theory and practice of size exclusion chromatography for the analysis of protein aggregates. J. Pharm. Biomed. Anal. 101, 161–173.

Fernandes, A.C., Gernaey, K. V, Krühne, U., 2018. "Connecting worlds – a view on microfluidics for a wider application." Biotechnol. Adv. 36, 1341–1366. doi: https://doi.org/10.1016/j.biotechadv.2018.05.001.

Fonseca, P., Vardaki, I., Occhionero, A., Panaretakis, T., 2016. Metabolic and signaling functions of cancer cell-derived extracellular vesicles. In: International Review of Cell and Molecular Biology. Elsevier, pp. 175–199.

Ford, T., Graham, J., Rickwood, D., 1994. Iodixanol: A Nonionic Iso-osmotic Centrifugation Medium for the Formation of Self-Generated Gradients. Anal. Biochem. https://doi.org/10.1006/abio.1994.1350.

Gallart-Palau, X., Serra, A., Wong, A.S.W., Sandin, S., Lai, M.K.P., Chen, C.P., Kon, O.L., Sze, S.K., 2015. Extracellular vesicles are rapidly purified from human plasma by PRotein Organic Solvent PRecipitation (PROSPR). Sci. Rep. 5, 14664.

Gallart-Palau, X., Serra, A., Sze, S.K., 2016. Enrichment of extracellular vesicles from tissues of the central nervous system by PROSPR. Mol. Neurodegener. 11, 41.

Gallo-Villanueva, R.C., Jesús-Pérez, N.M., Martínez-López, J.I., Pacheco, A., Lapizco-Encinas, B.H., 2011. Assessment of microalgae viability employing insulator-based dielectrophoresis. Microfluid. Nanofluid. 10, 1305–1315. https://doi.org/10.1007/s10404-010-0764-3.

Gao, F., Jiao, F., Xia, C., Zhao, Y., Ying, W., Xie, Y., Guan, X., Tao, M., Zhang, Y., Qin, W., Qian, X., 2019. A novel strategy for facile serum exosome isolation based on specific interactions between phospholipid bilayers and TiO2. Chem. Sci. 10, 1579–1588. https://doi.org/10.1039/c8sc04197k.

Gardiner, C., Di Vizio, D., Sahoo, S., Théry, C., Witwer, K.W., Wauben, M., Hill, A.F., 2016. Techniques used for the isolation and characterization of extracellular vesicles: results of a worldwide survey. J. Extracell. vesicles 5, 32945.

Gatti, S., Bruno, S., Deregibus, M.C., Sordi, A., Cantaluppi, V., Tetta, C., Camussi, G., 2011. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia–reperfusion-induced acute and chronic kidney injury. Nephrol. Dial. Transplant. 26, 1474–1483.

Gholizadeh, S., Draz, M.S., Zarghooni, M., Sanati-Nezhad, A., Ghavami, S., Shafiee, H., Akbari, M., 2017. Microfluidic approaches for isolation, detection, and characterization of extracellular vesicles: current status and future directions. Biosens. Bioelectron. 91, 588–605.

Gonzales, P.A., Pisitkun, T., Hoffert, J.D., Tchapyjnikov, D., Star, R.A., Kleta, R., Wang, N.S., Knepper, M.A., 2009. Large-scale proteomics and phosphoproteomics of urinary exosomes. J. Am. Soc. Nephrol. 20, 363–379.

Gossett, D.R., Tse, H.T.K., Dudani, J.S., Goda, K., Woods, T.A., Graves, S.W., Di Carlo, D., 2012. Inertial manipulation and transfer of microparticles across laminar fluid streams. Small 8, 2757–2764.

Grant, R., Ansa-Addo, E., Stratton, D., Antwi-Baffour, S., Jorfi, S., Kholia, S., Krige, L., Lange, S., Inal, J., 2011. A filtration-based protocol to isolate human plasma membrane-derived vesicles and exosomes from blood plasma. J. Immunol. Methods 371, 143–151.

Greening, D.W., Xu, R., Ji, H., Tauro, B.J., Simpson, R.J., 2015. A protocol for exosome isolation and characterization: evaluation of ultracentrifugation, density-gradient separation, and immunoaffinity capture methods. In: Proteomic Profiling. Springer, pp. 179–209.

Guo, S.C., Tao, S.C., Dawn, H., 2018. Microfluidics-based on-a-chip systems for isolating and analysing extracellular vesicles. J. Extracell. Vesicles 7. https://doi.org/10.1080/20013078.2018.1508271.

Guo, Q.R., Zhang, L.L., Liu, J.F., Li, Z., Li, J.J., Zhou, W.M., Wang, H., Li, J.Q., Liu, D.Y., Yu, X.Y., Zhang, J.Y., 2021. Multifunctional microfluidic chip for cancer diagnosis and treatment. Nanotheranostics 5, 73–89. https://doi.org/10.7150/ntno.49614.

Han, P., Bartold, P.M., Salomon, C., Ivanovski, S., 2020. Salivary small extracellular vesicles associated miRNAs in periodontal status—A pilot study. Int. J. Mol. Sci. 21, 1–13. https://doi.org/10.3390/ijms21082809.

Hannafon, B.N., Trigoso, Y.D., Calloway, C.L., Zhao, Y.D., Lum, D.H., Welm, A.L., Zhao, Z.J., Blick, K.E., Dooley, W.C., Ding, W.Q., 2016. Plasma exosome microRNAs are indicative of breast cancer. Breast Cancer Res. 18, 90.

Harrison, H., Lu, X., Patel, S., Thomas, C., Todd, A., Johnson, M., Raval, Y., Tzeng, T.R., Song, Y., Wang, J., Li, D., Xuan, X., 2015. Electrokinetic preconcentration of particles and cells in microfluidic reservoirs. Analyst 140, 2869–2875. https://doi.org/10.1039/c5an00105f.

He, M., Crow, J., Roth, M., Zeng, Y., Godwin, A.K., 2014. Integrated immunoisolation and protein analysis of circulating exosomes using microfluidic technology. Lab Chip. https://doi.org/10.1039/c4lc00662c.

Heath, N., Grant, L., De Oliveira, T.M., Rowlinson, R., Osteikoetxea, X., Dekker, N., Overman, R., 2018. Rapid isolation and enrichment of extracellular vesicle preparations using anion exchange chromatography. Sci. Rep. 8, 1–12.

Heinemann, M.L., Vykoukal, J., 2017. Sequential filtration: A gentle method for the isolation of functional extracellular vesicles. In: Extracellular Vesicles. Springer, pp. 33–41.

Heinemann, M.L., Ilmer, M., Silva, L.P., Hawke, D.H., Recio, A., Vorontsova, M.A., Alt, E., Vykoukal, J., 2014. Benchtop isolation and characterization of functional exosomes by sequential filtration. J. Chromatogr. A. https://doi.org/10.1016/j.chroma.2014.10.026.

Helwa, I., Cai, J., Drewry, M.D., Zimmerman, A., Dinkins, M.B., Khaled, M.L., Seremwe, M., Dismuke, W.M., Bieberich, E., Stamer, W.D., Hamrick, M.W., Liu, Y., 2017. A comparative study of serum exosome isolation using differential ultracentrifugation and three commercial reagents. PLoS One 12, 1–22. https://doi.org/10.1371/journal.pone.0170628.

Hisey, C.L., Dorayappan, K.D.P., Cohn, D.E., Selvendiran, K., Hansford, D.J., 2018. Microfluidic affinity separation chip for selective capture and release of label-free ovarian cancer exosomes. Lab Chip 18, 3144–3153. https://doi.org/10.1039/c8lc00834e.

Hong, P., Koza, S., Bouvier, E.S.P., 2012. A review size-exclusion chromatography for the analysis of protein biotherapeutics and their aggregates. J. Liq. Chromatogr. Relat. Technol. 35, 2923–2950.

Hoshino, A., Costa-Silva, B., Shen, T.-L., Rodrigues, G., Hashimoto, A., Mark, M.T., Molina, H., Kohsaka, S., Di Giannatale, A., Ceder, S., 2015. Tumour exosome integrins determine organotropic metastasis. Nature 527, 329–335.

Hu, J., Cui, X., Gong, Y., Xu, X., Gao, B., Wen, T., Lu, T.J., Xu, F., 2016. Portable microfluidic and smartphone-based devices for monitoring of cardiovascular diseases at the point of care. Biotechnol. Adv. 34, 305–320. https://doi.org/10.1016/j.biotechadv.2016.02.008.

Huang, L.R., Cox, E.C., Austin, R.H., Sturm, J.C., 2004. Continuous Particle Separation Through Deterministic Lateral Displacement. Science (80-.) 304, 987–990. https://doi.org/10.1126/science.1094567.

Ibsen, S.D., Wright, J., Lewis, J.M., Kim, S., Ko, S.Y., Ong, J., Manouchehri, S., Vyas, A., Akers, J., Chen, C.C., Carter, B.S., Esener, S.C., Heller, M.J., 2017. Rapid Isolation and Detection of Exosomes and Associated Biomarkers from Plasma. ACS Nano 11, 6641–6651. https://doi.org/10.1021/acsnano.7b00549.

Imai, A., Oka, S., Susuga, M., Tsutsui, N., Haga-Tsujimura, M., Saitoh, E., 2021. Comprehensive analysis and comparison of proteins in salivary exosomes of climacteric and adolescent females. Odontology 109, 82–102. https://doi.org/10.1007/s10266-020-00538-4.

Inan, H., Wang, S., Inci, F., Baday, M., Zangar, R., Kesiraju, S., Anderson, K.S., Cunningham, B.T., Demirci, U., 2017. Isolation, Detection, and Quantification of Cancer Biomarkers in HPV-Associated Malignancies. Sci. Rep. https://doi.org/10.1038/s41598-017-02672-6.

Inci, F., Celik, U., Turken, B., Özer, H.Ö., Kok, F.N., 2015. Construction of P-glycoprotein incorporated tethered lipid bilayer membranes. Biochem. Biophys. Reports. https://doi.org/10.1016/j.bbrep.2015.05.012.

Inci, F., Ozen, M.O., Saylan, Y., Miansari, M., Cimen, D., Dhara, R., Chinnasamy, T., Yuksekkaya, M., Filippini, C., Kumar, D.K., Calamak, S., Yesil, Y., Durmus, N.G., Duncan, G., Klevan, L., Demirci, U., 2018. A novel on-chip method for differential extraction of sperm in forensic cases. Adv. Sci. https://doi.org/10.1002/advs.201800121.

Inci, F., Saylan, Y., Kojouri, A.M., Ogut, M.G., Denizli, A., Demirci, U., 2020. A disposable microfluidic-integrated hand-held plasmonic platform for protein detection. Appl. Mater. Today. https://doi.org/10.1016/j.apmt.2019.100478.

Inci, F., Karaaslan, M.G., Gupta, R., Avadhani, A., Ogut, M.G., Atila, E.E., Duncan, G., Klevan, L., Demirci, U., 2021. Bio-inspired magnetic beads for isolation of sperm from heterogenous samples in forensic applications. Forensic Sci. Int. Genet. https://doi.org/10.1016/j.fsigen.2020.102451.

Iwai, K., Minamisawa, T., Suga, K., Yajima, Y., Shiba, K., 2016. Isolation of human salivary extracellular vesicles by iodixanol density gradient ultracentrifugation and their characterizations. J. Extracell. Vesicles 5, 1–17. https://doi.org/10.3402/jev.v5.30829.

Jeppesen, D.K., Hvam, M.L., Primdahl-Bengtson, B., Boysen, A.T., Whitehead, B., Dyrskjøt, L., Ørntoft, T.F., Howard, K.A., Ostenfeld, M.S., 2014. Comparative analysis of discrete exosome fractions obtained by differential centrifugation. J. Extracell. vesicles 3, 25011.

Jeppesen, D.K., Fenix, A.M., Franklin, J.L., Higginbotham, J.N., Zhang, Q., Zimmerman, L.J., Liebler, D.C., Ping, J., Liu, Q., Evans, R., Fissell, W.H., Patton, J.G., Rome, L.H., Burnette, D.T., Coffey, R.J., 2019. Reassessment of exosome composition. Cell 177, 428–445 e18. https://doi.org/10.1016/j.cell.2019.02.029.

Jeurissen, S., Vergauwen, G., Van Deun, J., Lapeire, L., Depoorter, V., Miinalainen, I., Sormunen, R., Van den Broecke, R., Braems, G., Cocquyt, V., Denys, H., Hendrix, A., 2017. The isolation of morphologically intact and biologically active extracellular vesicles from the secretome of cancer-associated adipose tissue. Cell Adhes. Migr. 11, 196–204. https://doi.org/10.1080/19336918.2017.1279784.

Ji, Q., Liu, J., Dong, Y., Wang, L., Dong, K., Setiz, B., Szentmáry, N., Qu, B., Shi, L., 2021. Exosomes derived from thymic stromal lymphopoietin-treated dendritic cells regulate T helper 17/regulatory T cell differentiation via miR-21/Smad7 axis. Exp. Cell Res. 398 https://doi.org/10.1016/j.yexcr.2020.112393.

Johnstone, R.M., Adam, M., Hammond, J.R., Orr, L., Turbide, C., 1987. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J. Biol. Chem. 262, 9412–9420.

Kalra, H., Adda, C.G., Liem, M., Ang, C., Mechler, A., Simpson, R.J., Hulett, M.D., Mathivanan, S., 2013. Comparative proteomics evaluation of plasma exosome isolation techniques and assessment of the stability of exosomes in normal human blood plasma. Proteomics 13, 3354–3364.

Kamerkar, S., LeBleu, V.S., Sugimoto, H., Yang, S., Ruivo, C.F., Melo, S.A., Lee, J.J., Kalluri, R., 2017. Exosomes facilitate therapeutic targeting of oncogenic KRAS in pancreatic cancer. Nature 546, 498–503.

Kanwar, S.S., Dunlay, C.J., Simeone, D.M., Nagrath, S., 2014. Microfluidic device (ExoChip) for on-chip isolation, quantification and characterization of circulating exosomes. Lab Chip 14, 1891–1900. https://doi.org/10.1039/c4lc00136b.

Keller, S., König, A.-K., Marmé, F., Runz, S., Wolterink, S., Koensgen, D., Mustea, A., Sehouli, J., Altevogt, P., 2009. Systemic presence and tumor-growth promoting effect of ovarian carcinoma released exosomes. Cancer Lett. 278, 73–81.
Keller, S., Ridinger, J., Rupp, A.-K., Janssen, J.W.G., Altevogt, P., 2011. Body fluid derived exosomes as a novel template for clinical diagnostics. J. Transl. Med. 9, 86.
Kim, D., Nishida, H., An, S.Y., Shetty, A.K., Bartosh, T.J., Prockop, D.J., 2016. Chromatographically isolated CD63+ CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. Proc. Natl. Acad. Sci. 113, 170–175.
Kim, Y.B., Yang, J.S., Lee, G. Bin, Moon, M.H., 2020. Evaluation of exosome separation from human serum by frit-inlet asymmetrical flow field-flow fractionation and multiangle light scattering. Anal. Chim. Acta 1124, 137–145. https://doi.org/10.1016/j.aca.2020.05.031.
Kim, K., Park, Jungjae, Jung, J., Lee, R., Park, Ji-ho, Yuk, J.M., Kim, K., Jung, J., 2021. Cyclic tangential flow filtration system for isolation of extracellular vesicles Cyclic tangential flow filtration system for isolation of extracellular vesicles 016103, pp. 0–10. https://doi.org/10.1063/5.0037768.
Koliha, N., Wiencek, Y., Heider, U., Jüngst, C., Kladt, N., Krauthäuser, S., Johnston, I.C. D., Bosio, A., Schauss, A., Wild, S., 2016. A novel multiplex bead-based platform highlights the diversity of extracellular vesicles. J. Extracell. Vesicles 5. https://doi.org/10.3402/jev.v5.29975.
Konoshenko, M.Y., Lekchnov, E.A., Vlassov, A.V., Laktionov, P.P., 2018. Isolation of extracellular vesicles: general methodologies and latest trends. Biomed. Res. Int. 2018.
Kowal, J., Arras, G., Colombo, M., Jouve, M., Morath, J.P., Primdal-Bengtson, B., Dingli, F., Loew, D., Tkach, M., Théry, C., 2016. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc. Natl. Acad. Sci. U. S. A. 113, E968–E977. https://doi.org/10.1073/pnas.1521230113.
Lagoa, R., Silva, J., Rodrigues, J.R., Bishayee, A., 2020. Advances in phytochemical delivery systems for improved anticancer activity. Biotechnol. Adv. 38, 107382. https://doi.org/10.1016/j.biotechadv.2019.04.004.
Lai, R.C., Yeo, R.W.Y., Tan, K.H., Lim, S.K., 2013. Exosomes for drug delivery - A novel application for the mesenchymal stem cell. Biotechnol. Adv. 31, 543–551. https://doi.org/10.1016/j.biotechadv.2012.08.008.
Lane, R.E., Korbie, D., Anderson, W., Vaidyanathan, R., Trau, M., 2015. Analysis of exosome purification methods using a model liposome system and tunable-resistive pulse sensing. Sci. Rep. 5, 7639.
Laurell, T., Petersson, F., Nilsson, A., 2007. Chip integrated strategies for acoustic separation and manipulation of cells and particles. Chem. Soc. Rev. 36, 492–506.
Lee, Y., El Andaloussi, S., Wood, M.J.A., 2012. Exosomes and microvesicles: extracellular vesicles for genetic information transfer and gene therapy. Hum. Mol. Genet. 21, R125–R134.
Lee, K., Shao, H., Weissleder, R., Lee, H., 2015. Acoustic purification of extracellular microvesicles. ACS Nano. https://doi.org/10.1021/nn506538f.
Lee, H., Zhang, D., Zhu, Z., Dela Cruz, C.S., Jin, Y., 2016. Epithelial cell-derived microvesicles activate macrophages and promote inflammation via microvesicle-containing microRNAs. Sci. Rep. 6 https://doi.org/10.1038/srep35250.
Lee, H., Zhang, D., Laskin, D.L., Jin, Y., 2018. Functional Evidence of Pulmonary Extracellular Vesicles in Infectious and Noninfectious Lung Inflammation. J. Immunol. 201, 1500–1509. https://doi.org/10.4049/jimmunol.1800264.
Lee, H., Groot, M., Pinilla-Vera, M., Fredenburgh, L.E., Jin, Y., 2019. Identification of miRNA-rich vesicles in bronchoalveolar lavage fluid: Insights into the function and heterogeneity of extracellular vesicles. J. Control. Release 294, 43–52. https://doi.org/10.1016/j.jconrel.2018.12.008.
Lewis, J.M., Vyas, A.D., Qiu, Y., Messer, K.S., White, R., Heller, M.J., 2018. Integrated Analysis of Exosomal Protein Biomarkers on Alternating Current Electrokinetic Chips Enables Rapid Detection of Pancreatic Cancer in Patient Blood. ACS Nano 12, 3311–3320. https://doi.org/10.1021/acsnano.7b08199.
Li, P., Mao, Z., Peng, Z., Zhou, L., Chen, Y., Huang, P.-H., Truica, C.I., Drabick, J.J., El-Deiry, W.S., Dao, M., 2015. Acoustic separation of circulating tumor cells. Proc. Natl. Acad. Sci. 112, 4970–4975.
Li, S., Ren, L., Huang, P.-H., Yao, X., Cuento, R.A., McCoy, J.P., Cameron, C.E., Levine, S. J., Huang, T.J., 2016. Acoustofluidic transfer of inflammatory cells from human sputum samples. Anal. Chem. 88, 5655–5661.
Li, A., Zhang, T., Zheng, M., Liu, Y., Chen, Z., 2017a. Exosomal proteins as potential markers of tumor diagnosis. J. Hematol. Oncol. 10, 175.
Li, P., Kaslan, M., Lee, S.H., Yao, J., Gao, Z., 2017b. Progress in exosome isolation techniques. Theranostics 7, 789.
Li, C., Lv, Y., Shao, C., Chen, C., Zhang, T., Wei, Y., Fan, H., Lv, T., Liu, H., Song, Y., 2019. Tumor-derived exosomal lncRNA GAS5 as a biomarker for early-stage non-small-cell lung cancer diagnosis. J. Cell. Physiol. 234, 20721–20727.
Li, B., Zang, G., Zhong, W., Chen, R., Zhang, Y., Yang, P., Yan, J., 2020a. Activation of CD137 signaling promotes neointimal formation by attenuating TET2 and transferrring from endothelial cell-derived exosomes to vascular smooth muscle cells. Biomed. Pharmacother. 121 https://doi.org/10.1016/j.biopha.2019.109593.
Li, F., You, M., Li, S., Hu, J., Liu, C., Gong, Y., Yang, H., Xu, F., 2020b. Paper-based point-of-care immunoassays: Recent advances and emerging trends. Biotechnol. Adv. 39, 107442. https://doi.org/10.1016/j.biotechadv.2019.107442.
Liang, K., Liu, F., Fan, J., Sun, D., Liu, C., Lyon, C.J., Bernard, D.W., Li, Y., Yokoi, K., Katz, M.H., 2017a. Nanoplasmonic quantification of tumour-derived extracellular vesicles in plasma microsamples for diagnosis and treatment monitoring. Nat. Biomed. Eng. 1, 1–11.
Liang, L.-G., Kong, M.-Q., Zhou, S., Sheng, Y.-F., Wang, P., Yu, T., Inci, F., Kuo, W.P., Li, L.-J., Demirci, U., Wang, S., 2017b. An integrated double-filtration microfluidic device for isolation, enrichment and quantification of urinary extracellular vesicles for detection of bladder cancer. Sci. Rep. 7, 46224. https://doi.org/10.1038/srep46224.
Liang, L.G., Sheng, Y.F., Zhou, S., Inci, F., Li, L., Demirci, U., Wang, S.Q., 2017c. An Integrated Double-Filtration Microfluidic Device for Detection of Extracellular Vesicles from Urine for Bladder Cancer Diagnosis. Methods Mol. Biol. https://doi.org/10.1007/978-1-4939-7253-1_29.
Liu, C., Guo, J., Tian, F., Yang, N., Yan, F., Ding, Y., Wei, J., Hu, G., Nie, G., Sun, J., 2017a. Field-free isolation of exosomes from extracellular vesicles by microfluidic viscoelastic flows. ACS Nano 11, 6968–6976.
Liu, F., Vermesh, O., Mani, V., Ge, T.J., Madsen, S.J., Sabour, A., Hsu, E.-C., Gowrishankar, G., Kanada, M., Jokerst, J. V, 2017b. The exosome total isolation chip. ACS Nano 11, 10712–10723.
Liu, D.S.K., Upton, F.M., Rees, E., Limb, C., Jiao, L.R., Krell, J., Frampton, A.E., 2020. Size-exclusion chromatography as a technique for the investigation of novel extracellular vesicles in cancer. Cancers (Basel). 12, 1–19. https://doi.org/10.3390/cancers12113156.
Livshits, M.A., Khomyakova, E., Evtushenko, E.G., Lazarev, V.N., Kulemin, N.A., Semina, S.E., Generozov, E.V., Govorun, V.M., 2015. Isolation of exosomes by differential centrifugation: Theoretical analysis of a commonly used protocol. Sci. Rep. 5, 17319.
Lobb, R.J., Becker, M., Wen, S.W., Wong, C.S.F., Wiegmans, A.P., Leimgruber, A., Möller, A., 2015. Optimized exosome isolation protocol for cell culture supernatant and human plasma. J. Extracell. Vesicles 4. https://doi.org/10.3402/jev.v4.27031.
Ludwig, N., Razzo, B.M., Yerneni, S.S., Whiteside, T.L., 2019. Optimization of cell culture conditions for exosome isolation using mini-size exclusion chromatography (mini-SEC). Exp. Cell Res. 378, 149–157. https://doi.org/10.1016/j.yexcr.2019.03.014.
Luga, V., Zhang, L., Viloria-Petit, A.M., Ogunjimi, A.A., Inanlou, M.R., Chiu, E., Buchanan, M., Hosein, A.N., Basik, M., Wrana, J.L., 2012. Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. Cell 151, 1542–1556.
Mani, V., Paleja, B., Larbi, K., Kumar, P., Tay, J.A., Siew, J.Y., Inci, F., Wang, S.Q., Chee, C., Wang, Y.T., Demirci, U., De Libero, G., Singhal, A., 2016. Microchip-based ultrafast serodiagnostic assay for tuberculosis. Sci. Rep. https://doi.org/10.1038/srep35845.
Martínez-González, E., Brochado-Kith, Ó., Gómez-Sanz, A., Martín-Carbonero, L., Jimenez-Sousa, M.Á., Martínez-Román, P., Resino, S., Briz, V., Fernández-Rodríguez, A., 2020. Comparison of methods and characterization of small RNAs from plasma extracellular vesicles of HIV/HCV coinfected patients. Sci. Rep. 10, 1–13. https://doi.org/10.1038/s41598-020-67935-1.
Marzano, G., Chiriacò, M.S., Primiceri, E., Dell'Aquila, M.E., Ramalho-Santos, J., Zara, V., Ferramosca, A., Maruccio, G., 2020. Sperm selection in assisted reproduction: A review of established methods and cutting-edge possibilities. Biotechnol. Adv. 40, 107498. https://doi.org/10.1016/j.biotechadv.2019.107498.
Mata-Gomez, M.A., Perez-Gonzalez, V.H., Gallo-Villanueva, R.C., Gonzalez-Valdez, J., Rito-Palomares, M., Martinez-Chapa, S.O., 2016. Modelling of electrokinetic phenomena for capture of PEGylated ribonuclease A in a microdevice with insulating structures. Biomicrofluidics 10. https://doi.org/10.1063/1.4954197.
Mataji-Kojouri, A., Ozen, M.O., Shahabadi, M., Inci, F., Demirci, U., 2020. Entangled Nanoplasmonic Cavities for Estimating Thickness of Surface-Adsorbed Layers. ACS Nano. https://doi.org/10.1021/acsnano.0c02797.
Melo, S.A., Luecke, L.B., Kahlert, C., Fernandez, A.F., Gammon, S.T., Kaye, J., LeBleu, V. S., Mittendorf, E.A., Weitz, J., Rahbari, N., Reissfelder, C., Pilarsky, C., Fraga, M.F., Piwnica-Worms, D., Kalluri, R., 2015. Glypican-1 identifies cancer exosomes and detects early pancreatic cancer. Nature. https://doi.org/10.1038/nature14581.
Meng, L., Turner, A.P.F., Mak, W.C., 2020. Soft and flexible material-based affinity sensors. Biotechnol. Adv. 39 https://doi.org/10.1016/j.biotechadv.2019.05.004.
Merchant, M.L., Rood, I.M., Deegens, J.K.J., Klein, J.B., 2017. Isolation and characterization of urinary extracellular vesicles: implications for biomarker discovery. Nat. Rev. Nephrol. 13, 731–749.
Mincheva-Nilsson, L., Baranov, V., Nagaeva, O., Dehlin, E., 2016. Isolation and characterization of exosomes from cultures of tissue explants and cell lines. Curr. Protoc. Immunol. 115, 14.42. 1–14.42. 21.
Mohammadi, M., Madadi, H., Casals-Terré, J., Sellarès, J., 2015. Hydrodynamic and direct-current insulator-based dielectrophoresis (H-DC-iDEP) microfluidic blood plasma separation. Anal. Bioanal. Chem. 407, 4733–4744. https://doi.org/10.1007/s00216-015-8678-2.
Momen-Heravi, F., Balaj, L., Alian, S., Mantel, P.Y., Halleck, A.E., Trachtenberg, A.J., Soria, C.E., Oquin, S., Bonebreak, C.M., Saracoglu, E., Skog, J., Kuo, W.P., 2013. Current methods for the isolation of extracellular vesicles. Biol. Chem. https://doi.org/10.1515/hsz-2013-0141.
Mulder, M., Mulder, J., 1996. Basic principles of membrane technology. Springer science & business media.
Musante, L., Saraswat, M., Ravidá, A., Byrne, B., Holthofer, H., 2013. Recovery of urinary nanovesicles from ultracentrifugation supernatants. Nephrol. Dial. Transplant. 28 (6), 1425–1433.
Nabet, B.Y., Qiu, Y., Shabason, J.E., Wu, T.J., Yoon, T., Kim, B.C., Benci, J.L., DeMichele, A.M., Tchou, J., Marcotrigiano, J., 2017. Exosome RNA unshielding couples stromal activation to pattern recognition receptor signaling in cancer. Cell 170, 352–366 e13.
Nakai, W., Yoshida, T., Diez, D., Miyatake, Y., Nishibu, T., Imawaka, N., Naruse, K., Sadamura, Y., Hanayama, R., 2016. A novel affinity-based method for the isolation of highly purified extracellular vesicles. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep33935.
Nath, S., Pigula, M., Khan, A.P., Hanna, W., Ruhi, M.K., Dehkordy, F.M., Pushpavanam, K., Rege, K., Moore, K., Tsujita, Y., Conrad, C., Inci, F., del Carmen, M.G., Franco, W., Celli, J.P., Demirci, U., Hasan, T., Huang, H.-C., Rizvi, I.,

2020. Flow-induced Shear Stress Confers Resistance to Carboplatin in an Adherent Three-Dimensional Model for Ovarian. A Role for EGFR-Targeted Photoimmunotherapy Informed by Physical Stress. J. Clin. Med, Cancer. https://doi.org/10.3390/jcm9040924.

Neves, J.S., Perez, S.A.C., Spencer, L.A., Melo, R.C.N., Weller, P.F., 2009. Subcellular fractionation of human eosinophils: isolation of functional specific granules on isoosmotic density gradients. J. Immunol. Methods 344, 64–72.

Nieuwstadt, H.A., Seda, R., Li, D.S., Fowlkes, J.B., Bull, J.L., 2011. Microfluidic particle sorting utilizing inertial lift force. Biomed. Microdevices 13, 97–105.

Oeyen, E., Van Mol, K., Baggerman, G., Willems, H., Boonen, K., Rolfo, C., Pauwels, P., Jacobs, A., Schildermans, K., Cho, W.C., Mertens, I., 2018. Ultrafiltration and size exclusion chromatography combined with asymmetrical-flow field-flow fractionation for the isolation and characterisation of extracellular vesicles from urine. J. Extracell. Vesicles 7. https://doi.org/10.1080/20013078.2018.1490143.

Onódi, Z., Pelyhe, C., Nagy, C.T., Brenner, G.B., Almási, L., Kittel, Á., Manček-Keber, M., Ferdinandy, P., Buzás, E.I., Giricz, Z., 2018. Isolation of high-purity extracellular vesicles by the combination of iodixanol density gradient ultracentrifugation and bind-elute chromatography from blood plasma. Front. Physiol. 9, 1–11. https://doi.org/10.3389/fphys.2018.01479.

Paolini, L., Zendrini, A., Di Noto, G., Busatto, S., Lottini, E., Radeghieri, A., Dossi, A., Caneschi, A., Ricotta, D., Bergese, P., 2016. Residual matrix from different separation techniques impacts exosome biological activity. Sci. Rep. 6, 1–11. https://doi.org/10.1038/srep23550.

Pariset, E., Agache, V., Millet, A., 2017. Extracellular vesicles: isolation methods. Adv. Biosyst. 1, 1700040.

Park, J., Han, D.H., Park, J.K., 2020. Towards practical sample preparation in point-of-care testing: User-friendly microfluidic devices. Lab Chip 20, 1191–1203. https://doi.org/10.1039/d0lc00047g.

Patel, D.B., Santoro, M., Born, L.J., Fisher, J.P., Jay, S.M., 2018. Towards rationally designed biomanufacturing of therapeutic extracellular vesicles: impact of the bioproduction microenvironment. Biotechnol. Adv. 36, 2051–2059. https://doi.org/10.1016/j.biotechadv.2018.09.001.

Patel, G.K., Khan, M.A., Zubair, H., Srivastava, S.K., Khushman, M., Singh, S., Singh, A. P., 2019. Comparative analysis of exosome isolation methods using culture supernatant for optimum yield, purity and downstream applications. Sci. Rep. 9, 1–10. https://doi.org/10.1038/s41598-019-41800-2.

Popovic, M., Mazzega, E., Toffoletto, B., De Marco, A., 2018. Isolation of anti-extracellular vesicle single-domain antibodies by direct panning on vesicle-enriched fractions. Microb. Cell Factories 17, 6.

Posokhov, Y.O., Kyrychenko, A., 2013. Effect of acetone accumulation on structure and dynamics of lipid membranes studied by molecular dynamics simulations. Comput. Biol. Chem. 46, 23–31.

Pysher, M.D., Hayes, M.A., 2007. Electrophoretic and dielectrophoretic field gradient technique for separating bioparticles. Anal. Chem. 79, 4552–4557. https://doi.org/10.1021/ac070534j.

Raposo, G., Nijman, H.W., Stoorvogel, W., Liejendekker, R., Harding, C. V, Melief, C.J., Geuze, H.J., 1996. B lymphocytes secrete antigen-presenting vesicles. J. Exp. Med. 183, 1161–1172.

Rider, M.A., Hurwitz, S.N., Meckes, D.G., 2016. ExtraPEG: A polyethylene glycol-based method for enrichment of extracellular vesicles. Sci. Rep. 6, 1–14. https://doi.org/10.1038/srep23978.

Ruivo, C.F., Adem, B., Silva, M., Melo, S.A., 2017. The biology of cancer exosomes: insights and new perspectives. Cancer Res. 77, 6480–6488.

Runz, S., Keller, S., Rupp, C., Stoeck, A., Issa, Y., Koensgen, D., Mustea, A., Sehouli, J., Kristiansen, G., Altevogt, P., 2007. Malignant ascites-derived exosomes of ovarian carcinoma patients contain CD24 and EpCAM. Gynecol. Oncol. 107, 563–571.

Ryu, K.J., Lee, J.Y., Park, C., Cho, D., Kim, S.J., 2020. Isolation of small extracellular vesicles from human serum using a combination of ultracentrifugation with polymer-based precipitation. Ann. Lab. Med. 40, 253–258. https://doi.org/10.3343/alm.2020.40.3.277.

Salafi, T., Zeming, K.K., Zhang, Y., 2017. Advancements in microfluidics for nanoparticle separation. Lab Chip 17, 11–33. https://doi.org/10.1039/C6LC01045H.

Salafi, T., Zhang, Yi, Zhang, Yong, 2019. A Review on Deterministic Lateral Displacement for Particle Separation and Detection. Nano-Micro Letters. Springer Singapore. https://doi.org/10.1007/s40820-019-0308-7.

Samuel, M., Chisanga, D., Liem, M., Keerthikumar, S., Anand, S., Ang, C.S., Adda, C.G., Versteegen, E., Jois, M., Mathivanan, S., 2017. Bovine milk-derived exosomes from colostrum are enriched with proteins implicated in immune response and growth. Sci. Rep. 7, 1–10. https://doi.org/10.1038/s41598-017-06288-8.

Santana, S.M., Antonyak, M.A., Cerione, R.A., Kirby, B.J., 2014. Microfluidic isolation of cancer-cell-derived microvesicles from hetergeneous extracellular shed vesicle populations. Biomed. Microdevices 16, 869–877. https://doi.org/10.1007/s10544-014-9891-z.

Saylan, Y., Denizli, A., 2018. Molecular fingerprints of hemoglobin on a nanofilm chip. Sensors (Switzerland). https://doi.org/10.3390/s18093016.

Sharma, P., Ludwig, S., Muller, L., Hong, C.S., Kirkwood, J.M., Ferrone, S., Whiteside, T. L., 2018a. Immunoaffinity-based isolation of melanoma cell-derived exosomes from plasma of patients with melanoma. J. Extracell. Vesicles 7. https://doi.org/10.1080/20013078.2018.1435138.

Sharma, S., Zhuang, R., Long, M., Pavlovic, M., Kang, Y., Ilyas, A., Asghar, W., 2018b. Circulating Tumor Cell Isolation, Culture, and Downstream Molecular Analysis. Biotechnol. Adv. 36, 1063–1078. https://doi.org/10.1016/j.biotechadv.2018.03.007.

Shi, L., Rana, A., Esfandiari, L., 2018. A low voltage nanopipette dielectrophoretic device for rapid entrapment of nanoparticles and exosomes extracted from plasma of healthy donors. Sci. Rep. 8, 1–12. https://doi.org/10.1038/s41598-018-25026-2.

Sjoqvist, S., Otake, K., Hirozane, Y., 2020. Analysis of cerebrospinal fluid extracellular vesicles by proximity extension assay: A comparative study of four isolation kits. Int. J. Mol. Sci. 21, 1–16. https://doi.org/10.3390/ijms21249425.

Skottvoll, F.S., Berg, H.E., Bjørseth, K., Lund, K., Roos, N., Bekhradnia, S., Thiede, B., Sandberg, C., Vik-Mo, E.O., Roberg-Larsen, H., Nyström, B., Lundanes, E., Wilson, S. R., 2018. Comparison of ultracentrifugation and a commercial kit for isolation of exosomes derived from glioblastoma and breast cancer cells. bioRxiv 1–43. https://doi.org/10.1101/274910.

Slyusarenko, M., Nikiforova, N., Sidina, E., Nazarova, I., Egorov, V., Garmay, Y., Merdalimova, A., Yevlampieva, N., Gorin, D., Malek, A., 2021a. Formation and Evaluation of a Two-Phase Polymer System in Human Plasma as a Method for Extracellular Nanovesicle Isolation. Polymers (Basel) 13, 458. https://doi.org/10.3390/polym13030458.

Slyusarenko, M., Nikiforova, N., Sidina, E., Nazarova, I., Egorov, V., Garmay, Y., Merdalimova, A., Yevlampieva, N., Gorin, D., Malek, A., 2021b. Formation and evaluation of a two-phase polymer system in human plasma as a method for extracellular nanovesicle isolation. Polymers (Basel). 13, 1–16. https://doi.org/10.3390/polym13030458.

Smith, J.T., Wunsch, B.H., Dogra, N., Ahsen, M.E., Lee, K., Yadav, K.K., Weil, R., Pereira, M.A., Patel, J.V., Duch, E.A., Papalia, J.M., Lofaro, M.F., Gupta, M., Tewari, A.K., Cordon-Cardo, C., Stolovitzky, G., Gifford, S.M., 2018. Integrated nanoscale deterministic lateral displacement arrays for separation of extracellular vesicles from clinically-relevant volumes of biological samples. Lab Chip 18, 3913–3925. https://doi.org/10.1039/c8lc01017j.

Sódar, B.W., Kittel, Á., Pálóczi, K., Vukman, K.V., Osteikoetxea, X., Szabó-Taylor, K., Németh, A., Sperlágh, B., Baranyai, T., Giricz, Z., 2016. Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection. Sci. Rep. 6, 24316.

Song, Z., Mao, J., Barrero, R.A., Wang, P., Zhang, F., Wang, T., 2021. Development of a CD63 Aptamer for E ffi cient Cancer Immunochemistry and Immunoa ffi nity-Based 1–16.

Sreepadmanabh, M., Toley, B.J., 2018. Investigations into the cancer stem cell niche using in-vitro 3-D tumor models and microfluidics. Biotechnol. Adv. 36, 1094–1110. https://doi.org/10.1016/j.biotechadv.2018.03.009.

Srivastava, A., Amreddy, N., Pareek, V., Chinnappan, M., Ahmed, R., Mehta, M., Razaq, M., Munshi, A., Ramesh, R., 2020. Progress in extracellular vesicle biology and their application in cancer medicine. Wiley Interdiscip. Rev. Nanomedicine Nanobiotechnology 12, 1–20. https://doi.org/10.1002/wnan.1621.

Stainless, P., Microreactor, S., Di, U., 2019. Kinetics of Fischer – Tropsch Synthesis in a 3-D.

Stuendl, A., Kunadt, M., Kruse, N., Bartels, C., Moebius, W., Danzer, K.M., Mollenhauer, B., Schneider, A., 2016. Induction of α-synuclein aggregate formation by CSF exosomes from patients with Parkinson's disease and dementia with Lewy bodies. Brain 139, 481–494.

Sun, L., Zhu, W., Zhao, P., Wang, Q., Fan, B., Zhu, Y., Lu, Y., Chen, Q., Zhang, J., Zhang, F., 2020. Long noncoding RNA UCA1 from hypoxia-conditioned hMSC-derived exosomes: a novel molecular target for cardioprotection through miR-873-5p/XIAP axis. Cell Death Dis. 11 https://doi.org/10.1038/s41419-020-02783-5.

Szatanek, R., Baran, J., Siedlar, M., Baj-Krzyworzeka, M., 2015. Isolation of extracellular vesicles: Determining the correct approach (review). Int. J. Mol. Med. 36, 11–17. https://doi.org/10.3892/ijmm.2015.2194.

Taller, D., Richards, K., Slouka, Z., Senapati, S., Hill, R., Go, D.B., Chang, H.-C., 2015. On-chip surface acoustic wave lysis and ion-exchange nanomembrane detection of exosomal RNA for pancreatic cancer study and diagnosis. Lab Chip 15, 1656–1666.

Tan, S.K., Pastori, C., Penas, C., Komotar, R.J., Ivan, M.E., Wahlestedt, C., Ayad, N.G., 2018. Serum long noncoding RNA HOTAIR as a novel diagnostic and prognostic biomarker in glioblastoma multiforme. Mol. Cancer 17, 74.

Tang, H., Wu, Z., Zhang, J., Su, B., 2013. Salivary lncRNA as a potential marker for oral squamous cell carcinoma diagnosis. Mol. Med. Rep. 7, 761–766.

Tang, Y.T., Huang, Y.Y., Zheng, L., Qin, S.H., Xu, X.P., An, T.X., Xu, Y., Wu, Y.S., Hu, X. M., Ping, B.H., Wang, Q., 2017. Comparison of isolation methods of exosomes and exosomal RNA from cell culture medium and serum. Int. J. Mol. Med. 40, 834–844. https://doi.org/10.3892/ijmm.2017.3080.

Tauro, B.J., Greening, D.W., Mathias, R.A., Ji, H., Mathivanan, S., Scott, A.M., Simpson, R.J., 2012. Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes. Methods 56, 293–304.

Tauro, B.J., Greening, D.W., Mathias, R.A., Mathivanan, S., Ji, H., Simpson, R.J., 2013. Two distinct populations of exosomes are released from LIM1863 colon carcinoma cell-derived organoids. Mol. Cell. Proteomics 12, 587–598. https://doi.org/10.1074/mcp.M112.021303.

Tay, H.M., Kharel, S., Dalan, R., Chen, Z.J., Tan, K.K., Boehm, B.O., Loo, S.C.J., Hou, H. W., 2017. Rapid purification of sub-micrometer particles for enhanced drug release and microvesicles isolation. NPG Asia Mater. 9, e434. https://doi.org/10.1038/am.2017.175.

Taylor, D.D., Shah, S., 2015. Methods of isolating extracellular vesicles impact down-stream analyses of their cargoes. Methods. https://doi.org/10.1016/j.ymeth.2015.02.019.

Taylor, D.D., Zacharias, W., Gercel-Taylor, C., 2011. Exosome isolation for proteomic analyses and RNA profiling. Methods Mol. Biol. https://doi.org/10.1007/978-1-61779-068-3_15.

Taylor, D.D., Zacharias, W., Gercel-taylor, C., 2014. Chapter 15 Exosome Isolation for Proteomic Analyses. https://doi.org/10.1007/978-1-61779-068-3.

Théry, C., Zitvogel, L., Amigorena, S., 2002. Exosomes: composition, biogenesis and function. Nat. Rev. Immunol. 2, 569–579.

Théry, C., Ostrowski, M., Segura, E., 2009. Membrane vesicles as conveyors of immune responses. Nat. Rev. Immunol. 9, 581–593.
Torino, S., Corrado, B., Iodice, M., Coppola, G., 2018. Pdms-based microfluidic devices for cell culture. Inventions 3, 1–14. https://doi.org/10.3390/inventions3030065.
Torregrosa Paredes, P., Gutzeit, C., Johansson, S., Admyre, C., Stenius, F., Alm, J., Scheynius, A., Gabrielsson, S., 2014. Differences in exosome populations in human breast milk in relation to allergic sensitization and lifestyle. Allergy 69, 463–471.
Vader, P., Mol, E.A., Pasterkamp, G., Schiffelers, R.M., 2016. Extracellular vesicles for drug delivery. Adv. Drug Deliv. Rev. 106, 148–156.
Valadi, H., Ekstrom, K., Bossios, A., Sjostrand, M., Lee, J.J., Lotvall, J.O., 2007. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat. Cell Biol. 9, 654–659. https://doi.org/10.1038/ncb1596.
van der Pol, E., Boeing, A.N., Harrison, P., Sturk, A., Nieuwland, R., 2012. Classification, Functions, and Clinical Relevance of Extracellular Vesicles. Pharmacol. Rev. https://doi.org/10.1124/pr.112.005983.
Van Deun, J., Mestdagh, P., Sormunen, R., Cocquyt, V., Vermaelen, K., Vandesompele, J., Bracke, M., De Wever, O., Hendrix, A., 2014. The impact of disparate isolation methods for extracellular vesicles on downstream RNA profiling. J. Extracell. vesicles 3, 24858.
Van Veldhoven, P.P., Baumgart, E., Mannaerts, G.P., 1996. Iodixanol (Optiprep), an improved density gradient medium for the iso-osmotic isolation of rat liver peroxisomes. Anal. Biochem. 237, 17–23.
Vergauwen, G., Dhondt, B., Van Deun, J., De Smedt, E., Berx, G., Timmerman, E., Gevaert, K., Miinalainen, I., Cocquyt, V., Braems, G., 2017. Confounding factors of ultrafiltration and protein analysis in extracellular vesicle research. Sci. Rep. 7, 1–12.
Vlassov, A.V., Magdaleno, S., Setterquist, R., Conrad, R., 2012. Exosomes: current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim. Biophys. Acta (BBA)-General Subj. 1820, 940–948.
Vu, C.H.T., Lee, H.G., Chang, Y.K., Oh, H.M., 2018. Axenic cultures for microalgal biotechnology: Establishment, assessment, maintenance, and applications. Biotechnol. Adv. 36, 380–396. https://doi.org/10.1016/j.biotechadv.2017.12.018.
Wang, Z., Wu, H.J., Fine, D., Schmulen, J., Hu, Y., Godin, B., Zhang, J.X.J., Liu, X., 2013. Ciliated micropillars for the microfluidic-based isolation of nanoscale lipid vesicles. Lab Chip. https://doi.org/10.1039/c3lc41343h.
Wang, J., Yan, F., Zhao, Q., Zhan, F., Wang, R., Wang, L., Zhang, Y., Huang, X., 2017. Circulating exosomal miR-125a-3p as a novel biomarker for early-stage colon cancer. Sci. Rep. 7, 1–9. https://doi.org/10.1038/s41598-017-04386-1.
Wang, Q. Long, Zhuang, X.Y., Sriwastva, M.K., Mu, J., Teng, Y., Deng, Z., Zhang, L., Sundaram, K., Kumar, A., Miller, D., Yan, J., Zhang, H.G., 2018. Blood exosomes regulate the tissue distribution of grapefruit-derived nanovector via CD36 and IGFR1 pathways. Theranostics 8, 4912–4924. https://doi.org/10.7150/thno.27608.
Wang, Z., Li, F., Rufo, J., Chen, C., Yang, S., Li, L., Zhang, J., Cheng, J., Kim, Y., Wu, M., Abemayor, E., Tu, M., Chia, D., Spruce, R., Batis, N., Mehanna, H., Wong, D.T.W., Huang, T.J., 2020. Acoustofluidic Salivary Exosome Isolation: A Liquid Biopsy Compatible Approach for Human Papillomavirus–Associated Oropharyngeal Cancer Detection. J. Mol. Diagnostics 22, 50–59. https://doi.org/10.1016/j.jmoldx.2019.08.004.
Weng, Y., Sui, Z., Shan, Y., Hu, Y., Chen, Y., Zhang, L., Zhang, Y., 2016. Effective isolation of exosomes with polyethylene glycol from cell culture supernatant for in-depth proteome profiling. Analyst 141, 4640–4646. https://doi.org/10.1039/c6an00892e.
Whiteside, T.L., 2016. Tumor-derived exosomes and their role in cancer progression. In: Advances in Clinical Chemistry. Elsevier, pp. 103–141.
Witwer, K.W., Buzas, E.I., Bemis, L.T., Bora, A., Lässer, C., Lötvall, J., Nolte, E.N., Piper, M.G., Sivaraman, S., Skog, J., 2013. Standardization of sample collection, isolation and analysis methods in extracellular vesicle research. J. Extracell. vesicles 2.
Woo, H.-K., Sunkara, V., Park, J., Kim, T.-H., Han, J.-R., Kim, C.-J., Choi, H.-I., Kim, Y.-K., Cho, Y.-K., 2017. Exodisc for rapid, size-selective, and efficient isolation and analysis of nanoscale extracellular vesicles from biological samples. ACS Nano 11, 1360–1370.
Wu, M., Ouyang, Y., Wang, Z., Zhang, R., Huang, P.-H., Chen, C., Li, H., Li, P., Quinn, D., Dao, M., 2017. Isolation of exosomes from whole blood by integrating acoustics and microfluidics. Proc. Natl. Acad. Sci. 114, 10584–10589.
Wu, S.C., Kuo, P.J., Rau, C.S., Wu, Y.C., Wu, C.J., Lu, T.H., Lin, C.W., Tsai, C.W., Hsieh, C.H., 2021. Subpopulations of exosomes purified via different exosomal markers carry different microrna contents. Int. J. Med. Sci. 18, 1058–1066. https://doi.org/10.7150/ijms.52768.
Wunsch, B.H., Smith, J.T., Gifford, S.M., Wang, C., Brink, M., Bruce, R.L., Austin, R.H., Stolovitzky, G., Astier, Y., 2016. Nanoscale lateral displacement arrays for the separation of exosomes and colloids down to 20nm. Nat. Nanotechnol. https://doi.org/10.1038/nnano.2016.134.
Xavier, M., Oreffo, R.O.C., Morgan, H., 2016. Skeletal stem cell isolation: A review on the state-of-the-art microfluidic label-free sorting techniques. Biotechnol. Adv. 34, 908–923. https://doi.org/10.1016/j.biotechadv.2016.05.008.
Yamamoto, K.R., Alberts, B.M., Benzinger, R., Lawhorne, L., Treiber, G., 1970. Rapid bacteriophage sedimentation in the presence of polyethylene glycol and its application to large-scale virus purification. Virology 40, 734–744.
Yáñez-Mó, M., Siljander, P.R.-M., Andreu, Z., Bedina Zavec, A., Borràs, F.E., Buzas, E.I., Buzas, K., Casal, E., Cappello, F., Carvalho, J., 2015. Biological properties of extracellular vesicles and their physiological functions. J. Extracell. vesicles 4, 27066.
Yang, D., Zhang, W., Zhang, H., Zhang, F., Chen, L., Ma, L., Larcher, L.M., Chen, S., Liu, N., Zhao, Q., Tran, P.H.L., Chen, C., Veedu, R.N., Wang, T., 2020. Progress, opportunity, and perspective on exosome isolation - Efforts for efficient exosome-based theranostics. Theranostics 10, 3684–3707. https://doi.org/10.7150/thno.41580.
Yu, D., Wu, Y., Shen, H., Lv, M., Chen, W., Zhang, X., Zhong, S., Tang, J., Zhao, J., 2015. Exosomes in development, metastasis and drug resistance of breast cancer. Cancer Sci. 106, 959–964.
Yuan, D., Zhao, Q., Yan, S., Tang, S.-Y., Alici, G., Zhang, J., Li, W., 2018. Recent progress of particle migration in viscoelastic fluids. Lab Chip 18, 551–567.
Yuana, Y., Levels, J., Grootemaat, A., Sturk, A., Nieuwland, R., 2014. Co-isolation of extracellular vesicles and high-density lipoproteins using density gradient ultracentrifugation. J. Extracell. vesicles 3, 23262.
Zarovni, N., Corrado, A., Guazzi, P., Zocco, D., Lari, E., Radano, G., Muhhina, J., Fondelli, C., Gavrilova, J., Chiesi, A., 2015. Integrated isolation and quantitative analysis of exosome shuttled proteins and nucleic acids using immunocapture approaches. Methods 87, 46–58.
Zaveckas, M., Snipaitis, S., Pesliakas, H., Nainys, J., Gedvilaite, A., 2015. Purification of recombinant virus-like particles of porcine circovirus type 2 capsid protein using ion-exchange monolith chromatography. J. Chromatogr. B 991, 21–28.
Zeibi Shirejini, S., Mohammadi, A., 2017. Halogen-Lithium Exchange Reaction Using an Integrated Glass Microfluidic Device: An Optimized Synthetic Approach. Org. Process. Res. Dev. 21, 292–303. https://doi.org/10.1021/acs.oprd.6b00307.
Zeringer, E., Barta, T., Li, M., Vlassov, A. V, 2015. Strategies for isolation of exosomes. Cold Spring Harb Protoc 2015, pdb. top074476.
Zhan, Y., Du, L., Wang, Lishui, Jiang, X., Zhang, S., Li, J., Yan, K., Duan, W., Zhao, Y., Wang, Lili, 2018. Expression signatures of exosomal long non-coding RNAs in urine serve as novel non-invasive biomarkers for diagnosis and recurrence prediction of bladder cancer. Mol. Cancer 17, 142.
Zhang, P., He, M., Zeng, Y., 2016. Ultrasensitive microfluidic analysis of circulating exosomes using a nanostructured graphene oxide/polydopamine coating. Lab Chip 16, 3033–3042. https://doi.org/10.1039/c6lc00279j.
Zhao, Z., Yang, Y., Zeng, Y., He, M., 2016. A microfluidic ExoSearch chip for multiplexed exosome detection towards blood-based ovarian cancer diagnosis. Lab Chip 16, 489–496. https://doi.org/10.1039/c5lc01117e.
Zhao, R., Zhang, Yanli, Zhang, X., Yang, Y., Zheng, X., Li, X., Liu, Y., Zhang, Yi, 2018a. Exosomal long noncoding RNA HOTTIP as potential novel diagnostic and prognostic biomarker test for gastric cancer. Mol. Cancer 17, 1–5.
Zhao, Y., Weber, S.R., Lease, J., Russo, M., Siedlecki, C.A., Xu, L.-C., Chen, H., Wang, W., Ford, M., Simo, R., 2018b. Liquid biopsy of vitreous reveals an abundant vesicle population consistent with the size and morphology of exosomes. Transl. Vis. Sci. Technol. 7, 6.
Zheng, R., Du, M., Wang, X., Xu, W., Liang, J., Wang, W., Lv, Q., Qin, C., Chu, H., Wang, M., Yuan, L., Qian, J., Zhang, Z., 2018. Exosome-transmitted long non-coding RNA PTENP1 suppresses bladder cancer progression. Mol. Cancer 17, 1–13. https://doi.org/10.1186/s12943-018-0880-3.
Zhu, L., Qu, X.-H., Sun, Y.-L., Qian, Y.-M., Zhao, X.-H., 2014. Novel method for extracting exosomes of hepatocellular carcinoma cells. World J Gastroenterol: WJG 20, 6651.
Zhuo, S., Xun, M., Li, M., Kong, X., Shao, R., Zheng, T., Pan, D., Li, J., Li, Q., 2020. Rapid and label-free optical assay of S-layer protein with high sensitivity using TiO2-coated porous silicon-based microfluidic biosensor. Sensors Actuators B Chem. 321, 128524.