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Global trend in exosome isolation and application: an update concept in management of diseases

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Abstract

Extracellular vesicles (EVs) secreted by various cells offer great potential for use in the diagnosis and treatment of disease. EVs are heterogeneous membranous vesicles. Exosomes are a subtype of EVs, 40–150 nm spherical vesicles with a lipid layer derived from endosomes. Exosomes, which are involved in signal transduction and maintain homeostasis, are released from almost all cells, tissues, and body fluids. Although several methods exist to isolate and characterize EVs and exosomes, each technique has significant drawbacks and limitations that prevent progress in the field. New approaches in the biology of EVs show great potential for isolating and characterizing EVs, which will help us better understand their biological function. The strengths and limitations of conventional strategies and novel methods (microfluidic) for EV isolation are outlined in this review. We also present various exosome isolation techniques and kits that are commercially available and assess the global market demand for exosome assays.

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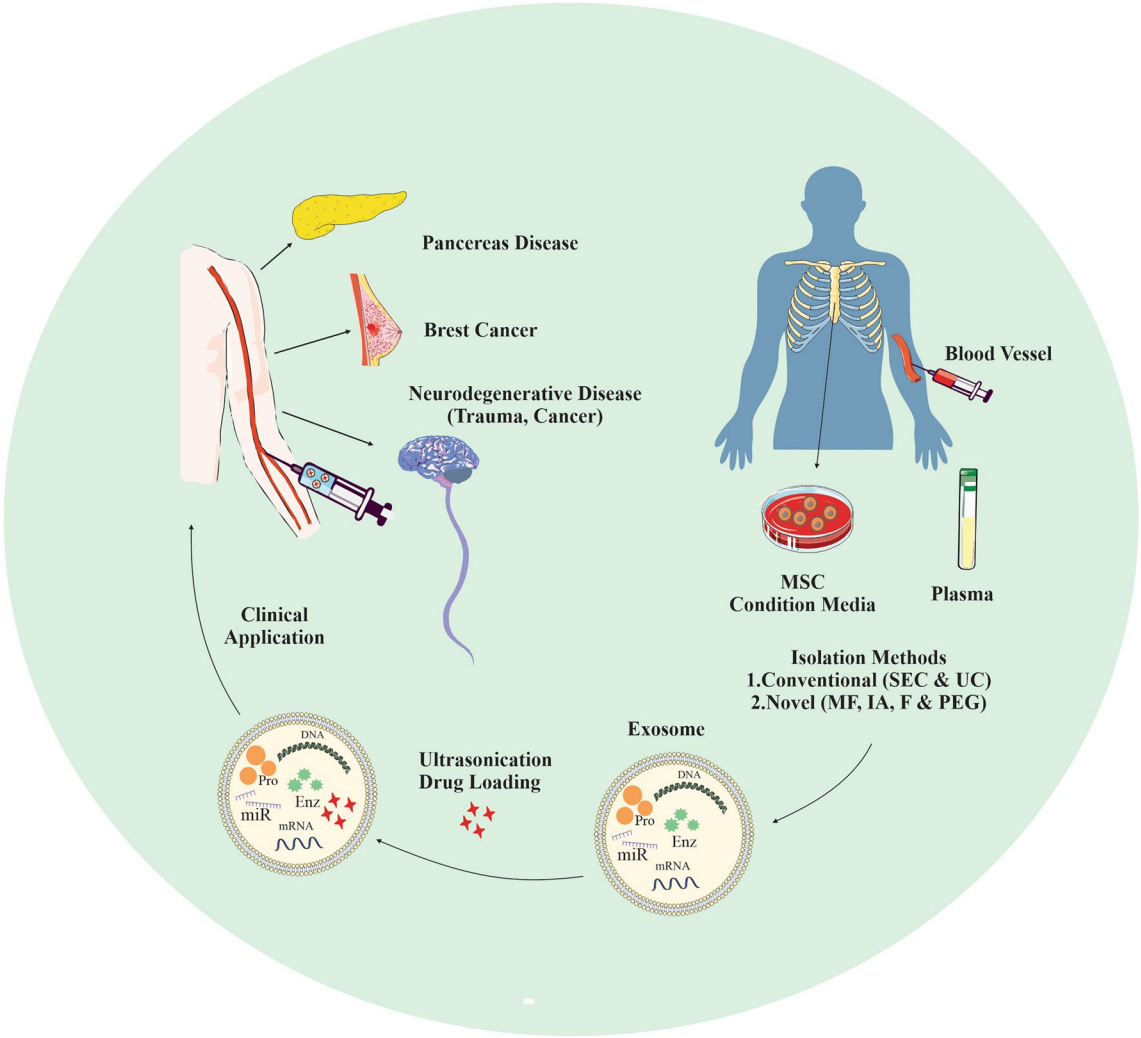
Graphical abstract



Keywords Isolation methods · Exosome · Extracellular vesicles · Microfluidics · Commercial kits

Introduction

Communication between cells in multicellular organisms involves the secretion of proteins that bind to receptors on neighboring cells and the release of membrane vesicles [1]. Membrane vesicles consist of a lipid bilayer containing soluble hydrophilic components and transmembrane proteins from the donor cell [2]. There are several types of secreted mentioned vesicles that have different biochemical and structural properties based on their intracellular site of origin, which likely influence their function. These include microvesicles, also called ectosomes, which range in size from 100 to 350 nm and are formed by vesicles that secrete directly from the plasma membrane [3, 4]. Apoptotic bodies are more large EVs than others that contain parts of dying cells, e.g., intact organelles, micronuclei, and chromatin remnants [5]. Amphisomes are intermediate organelles produced through the fusion of endosomes with autophagosomes within cells. Amphisome formation is an essential step during a sequential maturation process of autophagosomes before their ultimate fusion with lysosomes for cargo degradation [6]. Amphisomes form by single or multiple autophagosome-endosome fusions and can be recognized morphologically by their mixed autophagic-endocytic contents. Autophagy and exosome pathways are strictly interconnected at several levels. Amphisomes are then either degraded by lysosomal enzymes or released from the cell as exosomes.

Exosomes, extracellular vesicles with a diameter of 40–100 nm in which nucleic acids, proteins, and lipids

are embedded, regulate many pathophysiological processes, including immune responses, metabolism, etc. [7]. Exosomes can transport biochemically active molecules and regulate expression of gene and cellular functions [8]. Therefore, the pharmaceutical industry and academia are particularly interested in exosomes as an innovative drug delivery system.

Exosomes are constituted of several molecules, such as proteins, RNA, and DNA. Exosomal proteins differ in their properties from the cells or tissues from which they are derived [9]. Chaperones, adhesion molecules, and MHCs are the most important exosomal proteins [10]. Exosome-specific proteins could serve as biomarkers for exosome identification [11]. ALG -2-Interacting protein X (ALIX), Heat shock protein 70 (HSP 70), tetraspanins, and tumor susceptibility gene 101 (TSG101) are among the proteins with higher levels in exosomes [12]. Exosomes also contain mitochondrial DNA, noncoding RNAs (ncRNAs), and metabolic enzymes, as well as signaling molecules such as G-proteins and protein kinases [13]. Exosomes are taken up by recipient cells through multiple mechanisms, including interactions between exosomal molecules and recipient cells receptors. These include binding of phosphatidylserine to lymphocyte: T cell immunoglobulin domain and mucin domain protein 1 (TIM1) or TIM4, intercellular adhesion molecule 1 (ICAM1) to lymphocyte function-associated antigen 1 (LFA1), and possibly milk fat globule EGF factor 8 protein (MFGE8) to avβ3 or avβ5 integrins. After interaction with molecules on the recipient cell, the exosome could fuse directly to the membrane [14].

Cellular communication mediated by exosomes is involved in a variety of processes, including angiogenesis, senescence, immune signaling, differentiation, and proliferation. There is also evidence that exosomes aid in the spread of pathological proteins, including viral proteins and genomic RNA, microRNA, and mRNA found in exosomes from virus-infected cells. Exosomes can play an important role in viral infections, especially retroviruses, depending on their genetic content and proteins [15]. The ability of exosomes to transmit inflammatory mediators makes exosomes important players in inflammatory responses and in the pathogenesis of diseases such as type 2 diabetes mellitus, cancer, and degenerative diseases [16]. Since exosomes are abundant in biofluids, they have great potential as a non-invasive method to study exosome-associated biomarkers to determine their diagnostic and prognostic value. However, due to the challenges posed by the heterogeneity of extracellular vesicles, researchers are increasingly focusing on improving methods to isolate and characterize diverse populations of EVs. Here, we review current exosome isolation techniques, the diagnostic and therapeutic value of exosomes, and the commercial approach of exosome-based detection methods.

Preparation and characterization of exosomes

The "Minimal Information for Studies of Extracellular Vesicles" (MISEV) guidelines were created by the International Society for Extracellular Vesicles (ISEV) with the goal of standardizing the characterization, isolation, and reporting of EVs, such as exosomes. These guidelines provide researchers with a structured approach to ensure that their studies are both reproducible and comparable to others in the field. By following these guidelines, researchers can establish a solid framework for EVs research that promotes consistency and reliability [17].

A group of professors has formed the Extracellular Vesicles Track Consortium to establish standardized protocols for the isolation and characterization of EVs. The main goal of this group is to define best practices for EV research and to promote a collaborative approach to the field. Specifically, the consortium has developed a set of protocols for the isolation of exosomes from various biological fluids such as blood, urine, and cerebrospinal fluid. These protocols have been designed to be both reproducible and scalable, allowing researchers to isolate exosomes from both small and large volumes of biological samples [18].

Conventional approaches for exosome isolation

It is difficult to obtain exosomes in complex body fluids with a high yield because they are a nanoscale vesicular component. Exosomes are obtained from cell cultures or plasma, and their morphological and physical properties allow their identification [19]. Numerous techniques have been established for the isolation of exosomes. These techniques include differential and gradient density centrifugation, precipitation, immunoaffinity capture, filtration, size exclusion chromatography, and microfluidics-based techniques (Table 1).

Differential and gradient density centrifugation

The gold standard method for separating exosomes is centrifugation, especially ultracentrifugation [20]. These techniques do not require technical skill or complex sample preparation. Differential centrifugation typically requires several steps, including removal of cells, debris, and larger vesicles, followed by precipitation of exosomes at high speed at 100 000 g [21]. Density gradient ultracentrifugation (UC) is the other alternative technique that uses a density gradient for isolation. Sucrose is a commonly used medium for density centrifugation. In this method, vesicles are separated according to their flotation density, allowing them to float upward

Table 1 Conventional approaches for exosome isolation

Methods	Principle	Advantages	Disadvantages	References
Ultracentrifugation	Size-based separation	Easy to use, high capacity	Expensive, Time-consuming, exosome damage	[36]
Precipitation	Precipitation of low level solubility components of sample out of solution	Easy to use, cheap	Co-existence with microvesicles, lipoproteins, proteins, and precipitation reagents	[37]
Immunoaffinity	The specific binding between antibody and exosome-specific marker	High yield	Expensive, exosome damage, low yields	[37]
Filtration	Size-based separation	Easy to use, rapid, cheap	Exosome damage, loss of small size exosomes, co-existence with components	[38]
Size exclusion chromatography	Size-based separation, Polymer column filled with nanoporous beads	Maintain the integrity of exosomes, high yield, good reproducibility	Special equipment, co-isolation of albumin and lipoproteins	[39]

in a sucrose gradient. Therefore, exosomes can be separated without aggregates using this technique by pelleting and easily removing impurities at the bottom of the tube [7].

Size exclusion chromatography (SEC)

SEC is a size separation technique using a porous phase. Particles with a small radius can pass through the pores, while larger particles cannot enter the pores [22]. It has been reported that SEC indicates acceptable purity for the isolation of exosomes from blood [23]. SEC could be combined with other techniques such as precipitation to improve purity and yield. Although SEC preserves vesicle structure and integrity, it is not scalable for high-throughput applications due to its long run time [24].

Filtration

Filtration-based methods have recently become known for the isolation of exosomes [25]. Originally, filtration was used as a stand-alone technique, but recently the first two centrifugation steps have been replaced by filtration with ultracentrifugation [26]. Thus, filtration can remove large debris, while ultracentrifugation allows for greater purification of filtered samples. Although filtration is easier and faster than ultracentrifugation and does not require specialized equipment, it can affect exosomes by trapping them in the pores of the filters [27]. In addition, the force exerted as the sample passes over the filter membranes can cause structural damage to large vesicles [28].

Precipitation

This is a simple and instrument-free technique for the isolation of exosomes. Precipitation is a polymer-based technique in which the sample is mixed with a polymer at low temperature and adjusted salt concentration. Polyethylene glycol (PEG) is the most commonly used polymer in this procedure [29–31]. Commercial kits have been reported to be more effective in isolation than ultracentrifugation and nanomembrane concentrators [25]. PEG, however, carries the risk of adding impurities to the extracted exosomes that may interfere with their biological activities. Nowadays, a non-PEG/ exosome precipitation was developed by Invent Biotechnologies (MINUTETM, Plymouth, USA) [32].

Immune-affinity capture

Proteomics of exosomes has demonstrated the existence of several protein markers on the exosome membrane [33]. These proteins are the perfect markers for immunologic based isolation of exosomes because of the immunoaffinity interactions among the antibodies and proteins [34]. Selecting the correct and specific markers such as the tetraspanin family (CD9, CD63, and CD81) is the crucial step in immunoassay techniques and can be used for effective immunocapture [35].

Microfluidics for exosome isolation and analysis

Microfluidic devices can overcome the limitations of previous methods that do not provide the high levels of purity, recovery, and yield required for routine isolation and analysis in the clinic [40]. In addition, high cost, long processing times, and difficulties in standardization are other drawbacks. Microfluidics provides parallel separation and sensing capabilities for exosome isolation, detection, and analysis on a single chip. Fast performance, high specificity, high sensitivity, and a user-friendly format enable the production of point-of-care (POC) diagnostics for noninvasive liquid biopsy of exosomes for personalized medicine and clinical applications [41]. Generally, either labeled or label-free

methods are used in microfluidic systems. While label-based methods use immunoaffinity interaction to specifically separate exosomes from a mixture of other components, label-free methods use and isolate exosomes based on physicochemical differences such as size and density. Below, we present examples of each of these methods and discuss their biomedical potential for disease diagnosis, health monitoring, and therapy [42].

Label-free microfluidic methods for exosome isolation

There are several methods for sorting exosomes based on their density and size. For example, it has been reported that integrating acoustics with microfluidics results in high yield and pure exosome isolation directly from undiluted whole blood samples. By applying different acoustic forces to EVs depending on their size and density, the authors were able to separate particles of different sizes directly from whole blood [43] (Fig. 1). The cell removal unit of the microfluidic device was designed to fractionate blood components > with a diameter of 1 µm, including white blood cells (WBCs), red blood cells (RBCs), and platelets (PLTs), to obtain a cell-free plasma for the downstream exosome isolation unit that separates nanoscale bioparticles. This step requires the application of a higher frequency (~ 40 MHz). Finally, the products of the previous phase were delivered to the exosome isolation unit, which was able to discriminate between subgroups of EVs. Due to its biocompatible, label-free, and non-contact (automated) properties, such a method offers the potential to preserve the properties, structures, and functions of the isolated exosomes. Moreover, automated exosome isolation enables short processing times, biohazard containment, and provides reproducible results with convenient integration and minimal human intervention in downstream exosome analyzers [44].

Deterministic lateral displacement (DLD) column arrays are an additional label-free yet efficient technology for sorting, separation, and enrichment of micro- and nanoparticles. In this series, nano-DLD arrays are fabricated with constant slit sizes between 25 and 235 nm. At low Péclet numbers (Pe), where deterministic displacement and diffusion compete, nano-DLD arrays sort particles based on their size in the range of 20–110 nm with high resolution [45]. Another popular label-free detection method exploits the plasmon resonance properties of various novel materials to achieve nanoplasmonic phenomena. Light interacts uniquely with metallic nanoscale materials, such as gold nanoparticles, can provide unmatched sensitivity for real-time imaging and analysis [46]. Several methods use nanoplasmonic platforms to detect and characterize EVs. However, the most suitable formats are based on surface-enhanced Raman spectroscopy (SERS), surface plasmon resonance (SPR), and localized SPR [47]. Several nanoplasmon-based devices using SPR for sensing have been used for ultrasensitive label-free detection of exosomes [46, 48, 49]. Initially, imaging SPR (SPRi) was introduced as a label-free, quantitative, and real-time

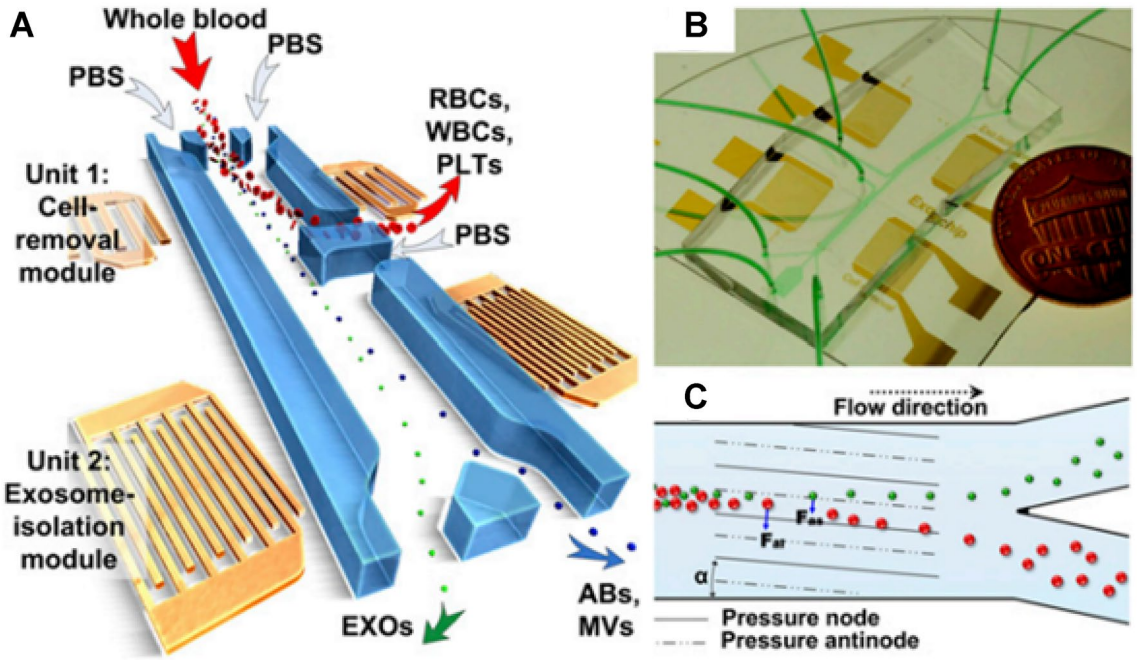


Fig. 1 schematic diagram of a label-free acoustofluidic device for exosome isolation. **A** A microfluidic device composed of different units. **B** An optical image of the integrated acoustofluidic device. **C** Working principle for size-based separation using lateral deflection induced by taSSAW field [44]

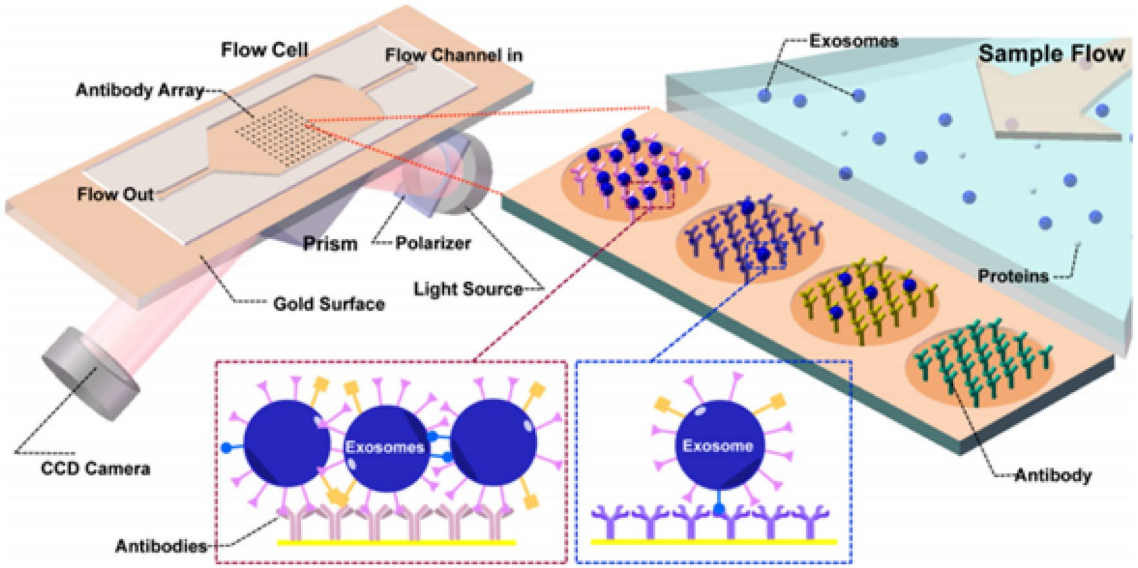


Fig. 2 An antiexosome antibody microarray is used in combination with SPRi to bind and detect exosomes in CCS. Once the sample is injected into the flow cell, the exosomes and antibodies can bind to the chip. The binding of a specific ligand to the coated antibody changes the refractive index, and the CCD camera records a higher intensity of the reflections [50]

method for the detection and characterization of tumor-emanating exosomes without purification or enrichment. Such SPR-based microfluidic device was demonstrated for the detection of exosomes in cell culture supernatant (CCS) of B16-F1/10 (mouse melanoma cell lines) and MHCC97H/L (human hepatoma cell lines) using antibodies for tyrosine kinase receptor MET, glycoprotein CD41b, and tetraspanins (e.g., CD9), specific antibodies against exosome transmembrane proteins. Moreover, SPR-based real-time monitoring of exosomes showed a positive relation in their abundance and metastatic ability in cell lines. The working principle of SPRi (Zhu et al., 2014) is shown in Fig. 2 [50].

Label-based (immunoaffinity)- microfluidics for exosome separation

Lable-based methods can be used to improve the specificity of exosomes for protein profiling. The markers are antigens selectively expressed on the surface of exosomes, such as CD63 and EpCAM. As mentioned previously, combining label-free detection methods such as SPR with label-based methods to isolate exosomes has yielded promising results [50].

In one preparation, an immunoaffinity-based microfluidic device led to the identification of more up to 20 proteins of exosome expressed in serous ovarian cancer (SOC) cell lines in compared with normal cells. In addition, HGF and STAT3 were identified as top regulatory proteins by pathway analysis. The discovery of novel exosome-based biomarkers can be used for the early detection of high grade SOC and the development of new targeted therapies that target high grade SOC-specific pathways, and thus may improve clinical outcomes in women with high grade SOC [51]. Microfluidic devices such as ExoChip [52] and ExoChip are reported for commercial purposes. While ExoChip uses anti-CD63, its new format uses both CD63 and phosphatidylserine (PS)-specific protein to increase the specificity of exosome separation. The device achieves 38% for healthy exosomes and 90% capture efficiency for cancer cell exosomes. It also separates 35% more A549 exosomes than the ExoChip. Moreover, the captured exosomes are readily released by Ca2 + chelation, which enables their downstream profiling [53]. Another device called ExoSearch uses immunomagnetic beads for multiplex measurement of EpCAM, CD24, and CA -125 exosomal tumor markers in plasma of ovarian cancer patients. The device showed significant diagnostic performance equivalent to the standard Bradford assay (Fig. 3) [54].

In another design, MVs were extracted from packed red blood cells (pRBCs) using a filter-assisted microfluidic device. MVs were labeled with anti-CD44, anti-CD47, and anti-CD55 magnetic nanoparticles, which allowed their further detection and quantification with a miniaturized nuclear magnetic resonance (μNMR) system. The results showed that a time-dependent increase in MV can be used as an effective measure of blood aging. In addition, the ability of MVs to act as generators of oxidative stress and consumers of nitric oxide became apparent. These new insights into the biology of MV can be used to improve transfusion safety and blood product quality [55].

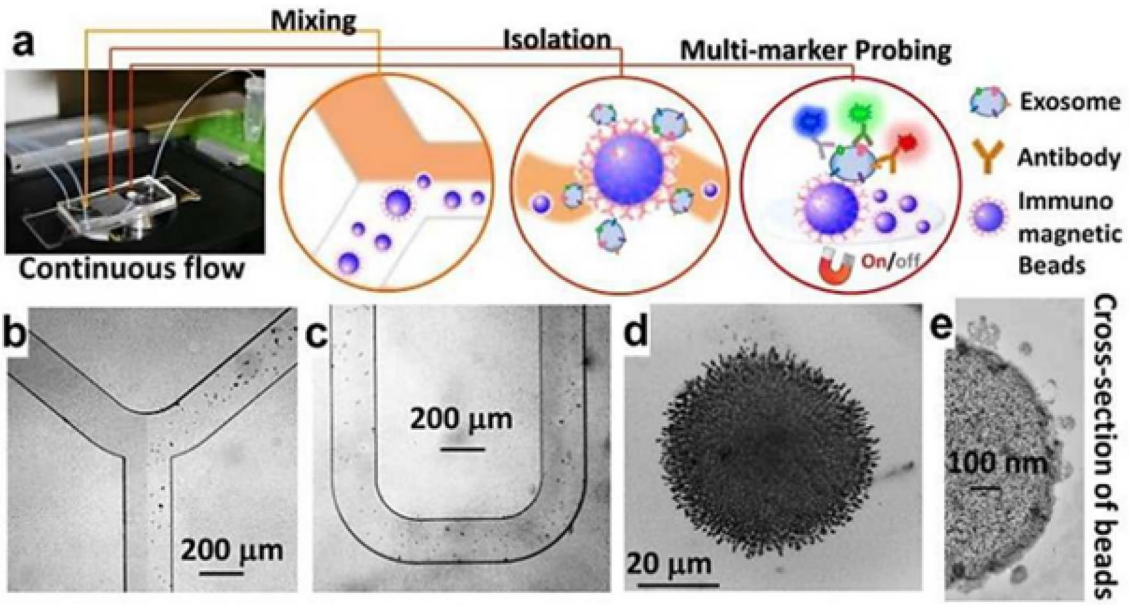


Fig. 3 **a** ExoSearch chip workflow for continuous exosome isolation, mixing, multiplexing, and in situ detection. The performance of immunomagnetic beads under a bright-field microscope (**b**–**c**). **d** Accumulation of exosome-bound immunomagnetic beads in a microchamber where exosomes are continuously collected and released by a magnet that can be switched on and off. e: Transmission electron microscope cross-section of an exosome-bound immunomagnetic bead [54]

In another nanointerface-based format, graphene oxide/polydopamine (GO /PDA) is used to improve the efficiency and specificity of exosome immuno-capturing. The nanointerface enabled an ultrasensitive exosome ELISA assay with a very low detection limit of 50 µL(-1) and a dynamic range of 4 log, which is significantly superior to existing methods. This platform differentiated healthy controls from ovarian cancer patients by quantitatively measuring exosomes from as little as 2 µl of plasma without sample processing [56]. Similarly, immunomagnetic beads immobilized with EpCAM antibodies are reported to provide highly accurate microvalve-based fluid control, allowing on-chip isolation and direct fluorescence-based detection of circulating exosomes within 1.5 h in the blood of breast cancer patients (56). Similar to µNMR [55], immunodetection based on CD24 and EpCAM markers is used in conjunction with a nano-plasmonic exosome assay (nPLEX) to improve sensitivity over previous methods. The device is portable when equipped with miniaturized optics and can collect exosomes for further study [57]. Microfluidics-based exosome sorting is also applied for real-time monitoring of drug efficacy in APNG (alkylpurine-DNA N-glycosylase) and MGMT (O(6)-methylguanine-DNA methyltransferase), the key enzymes that can repair temozolomide-induced DNA damage in glioblastoma multiforme (GBM). A microfluidic chip was used to study the mRNA levels of these enzymes in the tumor. The results show that the original tumor and exosomes have comparable mRNA levels for these two enzymes and that these levels change significantly during treatment. Consequently, such a platform, if validated in a large population, could be applied to GBM patients to predict drug response [58].

Characterization

According to the International Society for Extracellular Vesicles (ISEV), several characterization indices are required to identify isolated exosomes, including transmembrane or GPI-anchored proteins [59]. It is also possible to determine the purity of exosomes from biological fluids by detecting the presence or absence of certain non-EV structural proteins. Three steps are usually used for the identification of isolated exosomes in research: Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) for internal and external morphology, respectively; nanoparticle tracking analysis (NTA) and dynamic light scattering (DLS) for size; and Western blot, enzyme-linked immunosorbent assay (ELISA), and flow cytometry for surface protein markers. Methods for exosome characterization can be divided into external (particle sizing and morphology) and inclusion characterization (lipid raft and membrane protein) [59–64]. Table 2 provides an overview of the purpose, advantages, and disadvantages of the commonly used methods.

Table 2 Common exosome characterization methods

Methods	Detecting	Advantages	Disadvantages
TEM	Morphology	Observing internal and external structure of exosomes	Complicated operation, time concuming
NTA	Size and concentration	Fast, high resolution	Complicated operation
DLS	Size	10 nm detection limit	Not suitable for complex samples
Western blot	Marker proteins	Qualitative and quantitative analyzing	Complicated operation
Flow cytometry	Marker proteins	Multi-channel and high-throughput analyszing, fast, low sample requirement	Time-consuming, 400 nm detection limit, low accuracy and resolution
ELISA	Marker proteins	High specificity, rapid detection, high-throughput analyzing	Complicated operation, low repeatability

Diagnostics value of exosomes

Due to the functions of exosomes in disease progression and intercellular communication, as well as their cargo and surface proteins, they are currently used as diagnostic biomarkers in clinical trials. Exosomes have high plasma stability and can slow down the rapid degradation of nucleic acids [65]. Exosome-based liquid biopsy can be used to detect circulating tumor cells and their DNA or cell-free RNA from body fluids [66].

Cancer cell-derived exosomes contain a variety of proteins and RNAs that can serve as biomarkers for diagnosis and prognosis [67, 68]. In addition, exosomes are being used in the ongoing clinical investigation (NCT03478410) to characterize atrial fibrillation due to atherosclerosis, hypertension, and cardiac abnormalities [69]. Investigators will determine whether exosomes released from the epicardial adipose tissue of patients with and without atrial fibrillation differ as biomarkers of cardiac arrhythmias that can be used for both prevention and treatment.

Exosomes have been shown to have great potential as diagnostic biomarkers for neurodegenerative diseases such as Alzheimer's and Parkinson's disease [70]. Exosomes are involved in the pathogenesis of Parkinson's disease by transporting α-synuclein [71]. Mutations in Leucinerich repeat kinase 2 (LRRK2) are considered to be the cause of Parkinson's disease [72]. To develop a test to evaluate the effects of LRRK2 inhibitors, exosomal proteins, urinary and blood biomarkers from PD patients and healthy individuals were analyzed in a clinical trial (NCT01860118) [73]. As biomarkers, exosomes may also be involved in the development of Alzheimer's disease. In addition, exosome behavior in individuals at risk for AD is being investigated in two clinical trials (NCT03275363 and NCT03944603) [74, 75]. Changes in exosomal markers in blood and CSF at 2-year intervals in individuals aged 60 to 89 years are the primary outcome of the NCT03944603 study. By investigating the relationship between immune system biomarkers and aging, this study wants to find the mechanisms involved in mild cognitive impairment and Alzheimer's disease.

Exosomes have shown promise as diagnostic and prognostic biomarkers for a number of diseases. However, to obtain accurate data, it is critical to separate exosomal RNA from contaminating nanoparticles. Fortunately, several isolation and characterization techniques have been developed to effectively isolate exosomes from other extracellular vesicles and cell debris. However, obtaining reliable exosomal RNA in the clinical setting requires quality control measures and standardized protocols that are reproducible across laboratories.

Exosomes in the clinic

A lipid bilayer and abundance of adhesion molecules in exosomes make them ideal vehicles for targeted drug delivery. Low immunogenicity and the ability to cross the blood–brain barrier make them an ideal method for drug delivery [76]. Recent advances in nanomedicine led to the development of engineered exosomes, which demonstrated the potential of exosomes for targeted drug delivery [77]. Diabetes mellitus (DM) can be regulated by exosomes miRNAs, which are known to be key regulators of insulin resistance and pancreatic b-cell damage concerning the development of DM. This suggests that DM can be treated by exogenous silencing or activation of exosomal miRNAs [78].

Exosomal gp130 from breast cancer activates the signaling pathway of IL -6/STAT3 in macrophages, causing them to produce protumor cytokines and develop into a procancer phenotype. This discovery by Hamet et al. [79] raises the possibility that exosomal proteins, particularly breast cancer-derived exosomal proteins, are critical for cancer development. The researchers discovered that chemotherapy induces the EZH2/STAT3 signaling pathway in cancerous cells of breast and releases miR-378d and miR-378a-3p-rich exosomes that are taken up by patients. These exosomes activate the NOTCH and WNT stem cell pathways by targeting NAMB and DKK3, finally conclude in drug resistance. Consequently, avoiding exosomes during chemotherapy could minimize drug resistance [80].

As well, exosomes derived from bone marrow MSCs have been shown to modify the polarization of microglia to reduce demyelination and inflammation in rats [81]. Therefore, the administration of exosomes derived from bone marrow-derived MSCs has the potential to be a therapeutic strategy for the treatment of inflammatory diseases such as MS.

Exosomes have attracted much attention in the field of regenerative medicine because of their potential therapeutic applications. These tiny membrane vesicles have been found to promote tissue regeneration by stimulating cell proliferation and differentiation. In addition, exosomes possess immunomodulatory properties, making them a potential therapy for autoimmune diseases. Recently, ongoing clinical trials have demonstrated the safety and efficacy of mesenchymal stem cell-derived exosomes in patients with acute respiratory distress syndrome (ARDS) and have shown the potential of exosomes as a novel therapeutic strategy [82].

A recent Phase I/ II clinical trial has demonstrated the safety and efficacy of exosome therapy in patients with ischemic stroke [83]. In addition, a phase I clinical trial is investigating the potential use of dendritic cell-derived exosomes as a vaccine against melanoma [84]. The results of these studies could revolutionize the field of exosome therapy and remove current barriers to widespread use at the bedside.

Global market trends in exosome investigation

In recent years, an increase in exosome-related patents has been observed. Exosome products need to be produced at large scale and low cost to be used in commercial and clinical applications. The diagnostics segment is predicted to grow at a compound annual growth rate of 58.5%, from $10.0 million in 2016 to $100.0 million in 2021, which may indicate the importance of exosomes as diagnostic biomarkers in some diseases, particularly cancer. The therapeutics segment is forecast to grow 14.9%, from $5.0 million in 2016 to $10.0 million in 2021. This sluggishness could be due to the FDA's indecision on therapeutic authorities. However, stringent regulations and uncertainties in the therapeutic and biological definitions of EVs pose a challenge to the expanding EVs market. Overall, the global market for diagnostic and therapeutic exosome products is estimated to grow to $2.9 billion by 2030, with a projected CAGR of 29.4% between 2021 and 2030 [85]. Currently, there are several commercially developed methods for exosome enrichment and isolation. These techniques attempt to facilitate the isolation of exosomes that could be used for high-throughput biomarker studies [86]. Here, we comprehensively evaluate some companies and commercially available products for EVs (Table 3 and Table 4) [87].

Table 3 Exosome research and development of potential therapeutic commercial products are the focus of companies

Company	Source	Product	Potential	References
Kimera labs	Placental	XoGlo	Tissue repositioning to reduce scarring caused by healing	[88]
Capricor therapeutics	Cardiosphere-derived cells (CDCs)	CAP-2003	Examine the CDCs' anti-inflammatory, pro-angiogenic, antiapoptotic, and antifibrotic properties	[89]
NanoSomiX	Brain	ExoM and ExoC	Predictive biomarkers for possible nervous system disorders	[90]
Exosomedx	Plasma	ExoDx	Clinical laboratory improvement amendments (CLIA) certified product to conduct advanced clinical testing	[91]
Aruna biomedical	Neural	AB126	Murine thromboembolic models of stroke	[92]

Table 4 Commercially available products for exosome isolation

Product/company	Method	References
Exosome purification kit (Norgen Biotek), ExoQuick exosome precipitation (system biosciences), EXO-Prep (HasnaBioMed Life Sciences), Exo-spin (cell guidance systems), miRCURY (Exiqon), PureExo (101 Bio, Palo Alto), Invitrogen, RIBO	Precipitation	[93–98]
PURE-Evs (HansaBioMed), EVSecond (GL Sciences), qEV (iZON Science), ExoLutE (Rosetta exosome company)	Size-exclusion chromatography	[99–102]
Exosome isolation kit CD81/CD63 (Miltenyi Biotec), EpCAM (Thermofisher), MagCapture (FUJIFILM), Abcam	Immunoaffinity	[103–105]
ExoChip, ExoSearch	Microfluidic	[106]

Future outlook and conclusion

Exosomes have great potential as biomarkers for clinical applications in disease detection and therapy, as they not only possess signaling functions that enable communication between cells but can also be utilized for targeted drug delivery. However, their small size and heterogeneity present a challenge in isolating and detecting them at a low cost and with the desired sensitivity and selectivity in body fluids. Thus, it is crucial to develop an effective method to separate exosomes for clinical use. Conventional isolation methods have drawbacks, such as small sample volume, many processing steps, and structural damage to exosomes due to applied forces. In addition, commercially available exosome separation kits offer many advantages such as speed, high yield, and applicability, but they are also expensive, have low purity, and are not effective in separating exosomes from complex fluids. While conventional methods remain the gold standard for exosome separation, microfluidic methods reperesent a promising alternative that can overcome existing difficulties. Microfluidic devices also offer several advantages, such as low cost, relatively small size, low sample consumption, fast turnaround time, and high sensitivity, which make them suitable for clinical use, especially in the field of personalized medicine. Although several microfluidic-based isolation techniques have been developed for this purpose, it is difficult to apply these technologies on a large and industrial scale.

The choice of isolation method depends on the objective of the study and the availability of la-boratory equipment and resources. As mentioned earlier, various techniques have been used to separate different types of vesicles based on their size, shape, surface properties, and density, but inefficient separation methods, difficulties in characterization, and lack of specific bi-omarkers are still a matter of debate. On the other hand, it can be challenging to isolate exo-somes from amphisomes and ectosomes that have the same size and density [107]. To overcome these problems, it is important to carefully select appropriate controls and opti-mize experimental conditions to minimize contamination by other cellular components. The use of negative controls, such as detergent-treated samples or mock isolation procedures, can help identify potential sources of contamination. Optimization of isolation conditions such as buffer composition and centrifugation speed can also help improve the purity and specificity of isolated exosome preparations. Novel procedures for purification of autophagosomes and partial purification of amphisomes have allowed preliminary biochemical characterization of both organelles [108]. In addition, extensive studies are needed to confirm these properties in various disease con-texts. [11, 108].

It is expected that exosome research will continue to advance in the near future, which will likely lead to innovations in the treatment of patients. This review highlights recent scientific developments and technical obstacles in exosome isolation. It also presents a comprehensive analysis of current exosome products that provides recommendations for selecting the best commercial exosome kits based on the specific application.

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Data availability Enquiries about data availability should be directed to the authors.

Declarations

Conflict of interest This study was extracted from the dissertation registered at Tabriz University of Medical Sciences, Tabriz, Iran (Grant No: IR.TBZMED.VCR.REC.1398.241).

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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