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REVIEW

Recent advances in nanomaterial-based biosensors for the detection of exosomes

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Abstract

Exosomes are a type of extracellular vesicle actively secreted by almost all eukaryotic cells. They are ideal candidates for reliable next-generation biomarkers in the early diagnosis and therapeutic response evaluation of cancer. Thus, the quantification of exosomes is crucial in facilitating clinical research and application. Compared with traditional materials, nanomaterials have better optical, magnetic, electrical, and catalytic properties due to their small size, high specific surface area, and variable structure. The incorporation of nanomaterials into sensing systems is an attractive approach towards improving sensitivity and can provide improved sensor selectivity and stability. In this paper, we summarize the progress in nanomaterial-based exosome detection methods, including electrochemical biosensors, photoelectrochemical biosensors, colorimetric biosensors, fluorescence biosensors, chemiluminescence biosensors, electrochemiluminescence biosensors, surface plasmon resonance biosensors, and surface-enhanced Raman spectroscopy biosensors. Moreover, future research directions and challenges in exosome detection methods are discussed. We hope that this article will offer an overview of nanomaterial-based exosome detection techniques and open new avenues in disease research.

Keywords Nanomaterial · Exosome · Electrochemical biosensor · Optical biosensor

Introduction

With the worsening of the human living environment, cancer poses a serious threat to people's lives and health and has become a global public health issue [1]. The early diagnosis of cancer has become a pressing clinical issue. To date, tumor marker detection technology is nearly the only method and approach for early detection of asymptomatic microfocal tumors. The development of noninvasive detection methods for tumor markers is a research hotspot for the early diagnosis of cancer. Liquid biopsy is a noninvasive sampling method to obtain information about tumor cells based on novel biomarkers in circulation including circulating tumor cells, DNA, exosomes, and RNA. Liquid biopsy marks a great step forward in the road towards conquering tumors and opens a new horizon in cancer management.

Exosomes, a type of extracellular vesicle, are membranous vesicles with diameters ranging from 30 to 150 nm that contain lipids, proteins, and nucleic acids [2]. They have bilateral phospholipid membrane structures, can be secreted from all types of cells, and coexist in various body fluids, such as serum, urine, and saliva. In recent years, exosomes have been considered new biomarkers for tumor diagnosis because they can participate in intercellular communication (including proteins of specific diseases) and carry enriched proteins and nucleic acids from their parent cells [3]. They were first discovered by Trams in the 1980s in sheep erythrocytes cultured in vitro and they play a vital role in life activities [4]. For example, exosomes that originate from tumors play an important role in the formation, metabolism, and migration of tumors [5]. Exosomes derived from B cells or dendritic cells can play a role in adaptive immune regulation against pathogens or viruses [6]. Exosomes from HIV-1-infected cells increase the susceptibility of normal cells to HIV-1 [7]. Studies have confirmed that exosomes containing cancer cell information

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are secreted into the extracellular environment and can be used as specific biomarkers in the diagnosis and treatment of ovarian cancer [8], prostate cancer [9], liver cancer [10], and central nervous system diseases [11]. Therefore, exosome detection in body fluids has great potential to be applied as a noninvasive method of sampling and testing in clinical diagnosis [12].

Various approaches, including Western blotting [13], enzyme-linked immunosorbent assay (ELISA) [14], and mass spectrometry [15], have been applied to exosome detection in recent decades. Although these approaches are influential and widely used, they still have drawbacks and limitations. Nanoparticle tracking analysis (NTA) is the most widely used method used for the characterization of the concentration and size of exosomes [16]. However, it lacks the standardization required for exosome detection. NTA cannot distinguish the phenotypes present in a polydisperse sample [17]. Although transmission electron microscopy (TEM) and atomic force microscopy (AFM) can be used to study the morphology and size distribution of exosomes [18], microscopic methods do not have high-throughput or rapid detection capability. Therefore, scientists are committed to developing simple, sensitive, fast, high-throughput exosome detection methods. Under these circumstances, biosensors are a very good alternative for the detection of exosomes due to their efficiency, high selectivity, simple operation, and low cost.

Nanomaterials are materials with sizes and functions in the range of 1–100 nm in at least one dimension or that have structures composed of such materials as the basic units. Because of their excellent optical, electrical, magnetic, and catalytic properties, they are widely used in fields relating to the environment, food, and medicine to name but a few [19]. Following the rapid development of nanotechnology in recent years, its application in the field of biosensors has become a research hotspot. In the past decade, scientists have applied nanomaterials to the analysis and detection of exosomes to improve the accuracy, sensitivity, and speed of analysis and detection and to facilitate the development of rapid and simple exosome analysis methods, further providing technical support for the early diagnosis of cancer. Some reviews have summarized the progress in exosome biosensors and have briefly mentioned nanomaterials in exosome detection [20–26]. To the best of our knowledge, there has not been a comprehensive review of nanomaterial-based biosensors for the detection of exosomes. In this paper, we summarize the advances made since 2014 in nanomaterial-based biosensors for the detection of exosomes, systematically introducing electrochemical biosensors, photoelectrochemical biosensors, colorimetric biosensors, fluorescence biosensors, chemiluminescence biosensors, electrochemiluminescence biosensors, surface plasmon resonance biosensors, and surface-enhanced Raman scattering biosensors constructed from metal and metal oxide nanomaterials, quantum dots, metal-organic frameworks, and carbon nanomaterials, among others (Fig. 1). Future research directions and challenges in exosome detection methods are discussed.

Nanomaterial-based biosensor

This section highlights nanomaterial-based biosensor platforms that have been reported, such as electrochemical biosensors, photoelectrochemical biosensors, and colorimetric biosensors. Table 1 summarizes recent advances in nanomaterial-based biosensors used to determine exosomes. Magnetic nanoparticles, metal and metal oxide nanomaterials, metal-organic frameworks, quantum dots, and carbon nanomaterials have been introduced to improve biosensor performance. They have demonstrated great potential in cancer diagnosis.

Nanomaterial-based electrochemical biosensor

Due to the advantages of high sensitivity, good specificity, low cost, and easy integration, electrochemical biosensors are the most fascinating type of biosensors and are widely used in medical, environmental, food, and other fields [109]. The combination of nanomaterials with electrochemical sensors may provide a promising platform for the biosensing of exosomes. Nanomaterials can improve the performance of sensors by providing accelerated signal transduction and amplifying biorecognition events. To date, several nanomaterials, including magnetic nanoparticles, metal nanoparticles, quantum dots, polypyrrole nanotubes, metal-organic frameworks, and DNA nanomaterials, have been used to generate enhanced signals for electrochemical biosensors.

Magnetic nanoparticles

Magnetic nanoparticles can be enriched and separated by a simple external magnetic field. Thus, magnetic beads are often used in exosome detection. Wei et al. developed an electrochemical sensor for exosome protein detection [27]. First, exosomes isolated by magnetic bead–based technology were captured from serum or saliva by magnetic beads bound to antibodies (CD63) and enriched on electrodes by magnetic beads. Then, the exosome surface proteins were detected by the electrical signals generated by the antibody labeled with horseradish peroxidase. The sensitivity of this method is similar to that of Western blotting. In addition to the classic antigen-antibody interaction, aptamers are also an excellent choice due to their exosome binding affinity, small size, ease of synthesis, and stability. Zhou et al. constructed a magnetic bead–based electrochemical aptamer sensor, using a CD63 aptamer rather than CD63 antibody, that succeeded in detecting exosomes [28]. Exosomes were collected by ExoQuick-

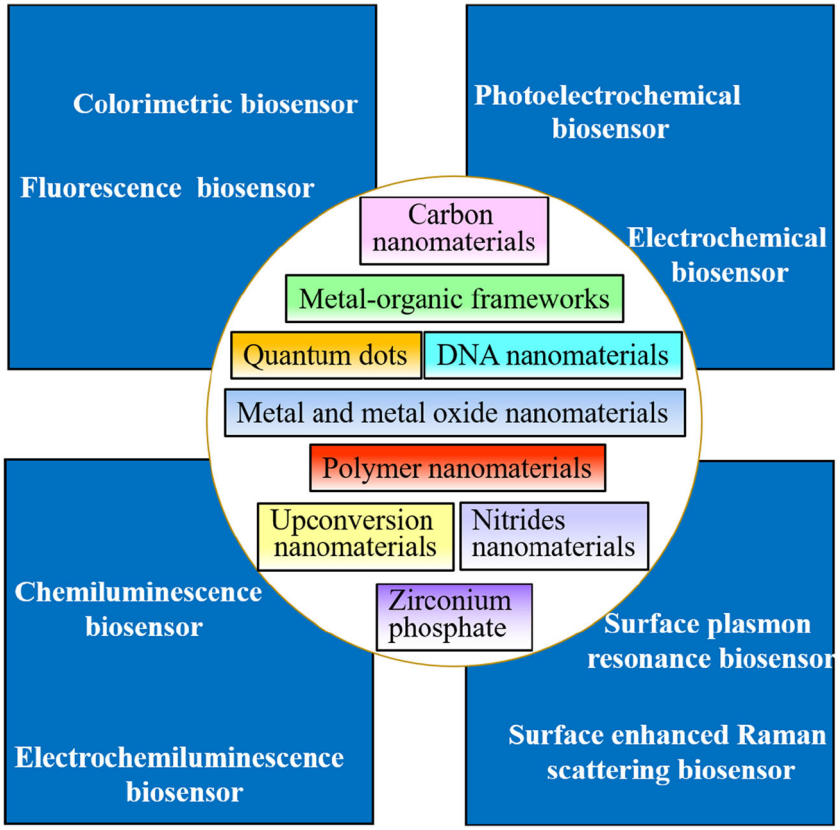

Fig. 1 Schematic representation of nanomaterial-based biosensors for the detection of exosomes

TC exosome precipitation reagent. The detection limit of this method was 10^6 particles/mL, which was 100 times lower than that of a commercial CD63 antibody–based immunoassay. However, it is difficult to accurately control the spatial orientation of single-stranded aptamers. Aptamers aggregate easily or can become entangled on electrodes, which hinders the combination of exosomes with the aptamers. To solve these problems, Wang et al. designed an aptamer sensor that incorporated amplified aptamers containing nucleotides into a DNA nanotetrahedron structure for electrochemical detection of cancer exosomes [29]. Exosomes were collected from the supernatant medium of HepG2 cells using conventional centrifugation. The oriented immobilization of aptamers significantly improved the accessibility of an artificial nucleobase-containing aptamer to the suspended exosomes, and the NTH-assisted aptasensor could detect exosomes with 100-fold higher sensitivity than the single-stranded aptamer-functionalized aptasensor. To amplify the signal, Miao et al. developed an ultrasensitive exosome electrochemical biosensor by incorporating the specific recognition of the CD63 aptamer, facile separation of magnetic Fe_3O_4@Au nanoparticles, and signal amplification of a multipedal DNA walker strategy [30]. Exosomes were collected by conventional centrifugation from HeLa cells. The detection limit of 6.0×10^3 particles/mL was obtained by testing exosomes in buffer solution (100 mM NaCl, 50 mM Tris-HCl, 10 mM $MgCl_2$, 100 μg/mL BSA, pH 7.9). This study sheds new light on DNA nanomachines and cancer diagnostics.

Metal nanoparticles

Metal nanoparticles, such as gold nanoparticles and silver nanoparticles, have an important place in the field of electrochemical biosensors due to their excellent electrochemical, electronic, and catalytic properties and good stability. Moreover, their surfaces are easy to functionalize. Thus, considerable effort has been devoted to metal nanoparticle–based electrochemical biosensors. Zhou et al. designed a microfabricated multichannel gold chip for quantitative exosome analysis. The expression of epithelial cell adhesion molecule (EPCAM) and prostate-specific membrane antigen (PSMA) was determined by the oxidation of silver nanoparticles and copper nanoparticles, respectively [31]. A limit of detection of 50 particles/mL was obtained (tested with 25-μL samples of exosomes extracted from PCa clinical samples in phosphate-buffered saline). It was found that the expression of EPCAM and PSMA on the surface of exosome from a prostate cancer patient was significantly higher than that on exosomes from healthy people. This electrochemical sensor provides a successful platform for protein marker detection from tumor-derived exosomes and microsomes and has the potential to monitor prostate cancer.

Table 1 Summary of nanomaterial-based biosensors used to detect exosomes

Device	Nanomaterial	Advantages	Limitations
Electrochemical biosensors	Magnetic nanoparticles [27–30] Metal nanoparticles [31] Quantum dots [32, 33] Polypyrrole nanotubes [34] Metal-organic frameworks [35, 36] DNA nanomaterials [37–41]	High sensitivity, rapid response, easily miniaturized devices, high selectivity	Low reproducibility, sample matrix effects
Photoelectrochemical biosensors	TiO_2 nanosilk@MoS_2 quantum dots [42]	Simple equipment, low price, high sensitivity, and easy miniaturization	Low reproducibility, sample matrix effects
Colorimetric biosensors	Gold nanoparticles [43–51] Carbon nanomaterials [52, 53] Metal oxides [54, 55]	Rapid response, simple operation, cost-effective system	Low sensitivity, limited multiplexing and quantification capability
Fluorescence biosensors	Quantum dots [56–58] Upconversion nanomaterials [59–61] Gold nanoparticles [62–66] 2D carbon nanomaterials [67–71] Metal oxides [72–74] Zirconium-phosphate [75] DNA nanomaterials [76]	High sensitivity, multiplexing capability	Expensive instruments, interference from background fluorescence and quenching effect
Chemiluminescence biosensors	Superparamagnetic iron oxide particles [77] CuS nanoparticles [78]	Simplicity, low cost, high sensitivity	Interference from background absorption
Electrochemiluminescence biosensors	Carbon-based nanomaterials [79–82] Quantum dots [83, 84]	High sensitivity, strong anti-interference capability, good selectivity, simple equipment, simple operation	Low reproducibility, sample matrix effects
Surface plasmon resonance biosensors	Gold nanoparticles [85–91] Titanium nitride nanomaterials [92] Magnetic nanomaterials [93]	High sensitivity, label-free, real time	Suffers from nonspecific adsorption
Surface-enhanced Raman scattering biosensors	Metal nanomaterials [94–108]	Superior sensitivity, complex biological sample applicability, nondestructive	Poor repeatability, high-cost equipment, data analysis

Quantum dots

Quantum dots are quasi-zero-dimensional semiconductor nanostructures that bind excitons in three spatial directions, and their quantum confinement effects result in good photoelectric properties. In recent years, the application of quantum dots in electrochemical sensors has gradually developed. After acid stripping pretreatment, electrochemical signals from quantum dots can be detected by anodic stripping voltammetry. Based on this principle, Boriachek et al. used CdSe quantum dots as signal-amplifying probes to construct electrochemical sensors for exosome detection [32]. First, exosomes isolated with total exosome isolation reagent were captured by magnetic nanoparticles modified with four-transmembrane protein antibodies. Then, through antigen-antibody binding, the antibodies specific for HER-2 and FAM134B on CdSe quantum dots were captured by exosomes secreted by breast and colon cancer cells. Dissolved in nitric acid, Cd^{2+} was quantitatively determined by anodic-stripping voltammetry. The detection limit of this method was 10^5 particles/mL, and the linear range was 10^5–10^{10} particles/mL (tested with 1-mL samples of exosomes extracted from cancer cells in culture medium). By using multiple quantum dots, electrochemical sensing for simultaneous detection of various tumor-specific exosomes could be developed. Then, they constructed electrochemical biosensors of miRNA-21 in exosomes using similar principles to achieve quantitative detection of exosomal miRNA-21 in serum [33]. The detection limit of this method was 1 pM, and the linear range was 1 pM–100 nM (tested with 25-μL samples of exosomes extracted from cells in culture medium). This method has potential significance in cancer screening, prognosis, and treatment.

Polymer nanomaterials

Polypyrrole is a typical conductive polymer with the advantages of good stability in various environmental conditions, high conductivity, and easy synthesis. Polypyrrole in micro/nanostructures has the advantages of both organic conductors and nanostructures and is widely applicable in the molecular wire, sensor, and biomedical fields. Lim et al. developed an electrochemical sensor based on self-supporting 3D polypyrrole nanotubes for effectively separating exosomes from complex samples and establishing quantitative analysis [34]. First, the surface of the conductive polypyrrole nanotubes was modified with a protein that can recognize the surface of the exosomes. When stimulated by electricity or glutathione, the captured exosomes could be well released from the surface of polypyrrole, so that electrochemical signals could be obtained. Vertically arranging the nanowires in a 3D structure effectively improved the exosome enrichment speed, which could be completed within 1 h.

Metal-organic frameworks

Metal-organic frameworks (MOFs) are a new type of material with the advantages of high specific surface areas, flexible porosity, chemical tenability, and durable sensing features. This makes them ideal materials in many fields, such as gas storage and separation, catalysis, sensing, drug release, and magnetism [110–112]. In terms of electrochemical sensing, MOFs not only provide more active sites for the reaction but also can be used as carriers to encapsulate nanoparticles to improve the catalytic activity of composite materials. Li et al. developed an electrochemical biosensor by using Zr-based metal-organic frameworks for the detection of glioblastoma-derived exosomes with practical applications [35]. Exosome pellets were isolated from U87 GBM cell supernatant by differential centrifugation. Zr-MOFs encapsulated with electroactive methylene blue could absorb on the surface of exosomes due to the interaction between Zr^{4+} and the intrinsic phosphate groups outside of exosomes. Thus, the concentration of exosomes (10 μL) in Tris-HCl (50 mM Tris and 150 mM NaCl, pH 7.4) could be directly quantified by monitoring the electroactive molecules inside MOFs, ranging from 9.5×10^6 to 1.9×10^{10} particles/mL, with a detection limit of 7.8×10^6 particles/mL. Moreover, this biosensor has been employed to analyze GBM-derived exosomes in human serum and to distinguish glioblastoma patients from healthy groups. To improve the sensitivity of exosome detection, a dual-signal self-calibration sensing platform was explored. Gui et al. developed a dual-signal and intrinsic self-calibration-specific cancer-derived exosome sensing platform based on black phosphorous nanosheets and MOFs [36]. The concentration of exosomes in PBS (1 mM, pH 7.4) could be quantified by monitoring the dual redox-signal responses of MB and Fc, ranging from 1.3×10^2 to 2.6×10^5 particles/mL, with a detection limit of 100 particles/mL. Moreover, it was used to detect exosomes in plasma samples of healthy individuals and breast cancer patients, revealing its significant potential for detection of specific cancer cell–derived exosomes.

DNA nanomaterials

DNA nanotechnology is a new technology for signal amplification with the advantages of predictability and programmability. It uses the molecular properties of DNA or other nucleic acids, such as self-assembly, to construct controllable novel nanoscale structures. Thanks to its good program controllability and biocompatibility, DNA nanotechnology has been widely applied in biosensors. Cao et al. established an electrochemical sensor based on signal amplification driven by a catalytic molecular machine for exosome detection [37]. First, exosomes isolated using an the exoEasy Maxi Kit were captured on the surface of immunomagnetic beads modified with

anti-CD63 antibody via an immunoaffinity reaction. It was subsequently identified by circular DNA containing the CD63 aptamer. After magnetic separation, the DNA could start the molecular catalytic machinery of the cascade toehold-mediated strand displacement reaction (CTSDR). Thanks to the high efficiency of molecular machines, a linear range from 1×10^5 to 5×10^7 particles/mL and a detection limit of 1.7×10^4 particles/mL were achieved (tested with 25-μL samples of exosomes secreted by HepG2 cells in culture medium, PBS). Huang et al. constructed an electrochemical sensor based on a hemin/G-quadruplex-assisted roll loop amplification signal amplification strategy to detect gastric cancer exosomes [38]. Exosomes derived from a human gastric cancer cell line were isolated by centrifugation. The limit of detection of exosomes derived from gastric cancer cells in 100 μL plasma samples was 9.5×10^2 particles/mL, and the linear range was 4.8×10^3–4.8×10^6 particles/mL. Dong et al. established an aptamer electrochemical sensor using the dual-signal amplification strategy of aptamer recognition inducing multiple DNA releases and cyclic enzyme amplification to realize sensitive detection of tumor exosomes derived from LNCaP cells in 10 μL RPMI-1640 medium (isolated with an Optima^{TM} XPN ultracentrifuge), with a limit of detection as low as 7.0×10^4 particles/mL [39]. Zhang et al. developed a ratio electrochemical biosensor based on the bipedal DNA "Walker" for detection of exosomal microRNA-21, as shown in Fig. 2 [40]. In the presence of miR-21, the DNA "Walker" was activated to walk continuously along the DNA track,

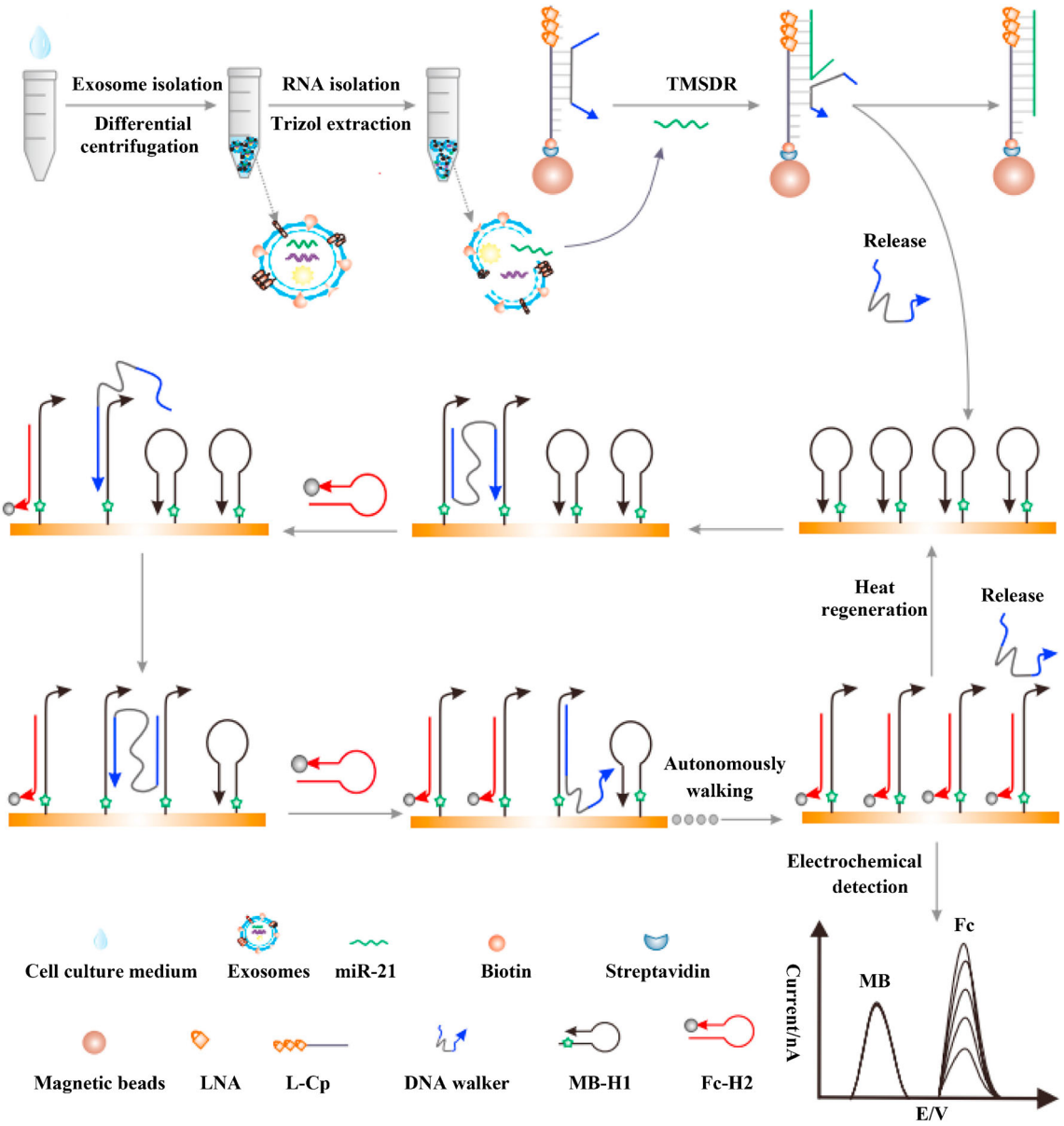

Fig. 2 Schematic illustration of the ratiometric electrochemical biosensor for exosomal miR-21 detection

resulting in conformational changes and a corresponding increase in the signal ratio generated by target-response and target-independent reporter molecules. With the cascade amplification of the DNA step-by-step signal, the biosensor exhibited superhigh sensitivity, and the limit of detection was as low as 67 aM (exosomes derived from breast cancer cell samples were isolated using the gold standard ultracentrifugation method and tested in 30 μL). Similarly, Zhao et al. developed an electrochemical ratiometric exosome biosensor based on a target-triggered 3D DNA walking machine and exonuclease III [41]. Exosomes derived from MCF-7 cells were isolated by centrifugation, and the detection limit of exosomes within 20 μL PBS was 1.3×10^4 particles/mL.

Nanomaterial-based photoelectrochemical biosensors

Photoelectrochemical biosensors developed based on electrochemical analysis are a new and rapidly developing analysis technology that combines photoelectric materials with biological analysis methods. Under illumination, the specific biorecognition between the recognition probe and the target identifier will cause the corresponding electrical signals to change. The relationship between the change in the electrical signal and the concentration of the target is the basis for qualitative and quantitative analysis via photoelectric analysis. Photoelectrochemical biosensors not only inherit the advantages of electrochemical analysis, including simple equipment, low price, high sensitivity, and easy miniaturization, but also have some unique advantages. Pang et al. constructed a self-driven sensor device based on TiO_2 nanosilk@MoS_2 quantum dots for detection of exosomal RNA [42]. MoS_2 quantum dots are aligned with heterojunction sensitized TiO_2 nanosilks by forming type I bands. The constructed device exhibits a stable and continuous energy output with an energy density as high as 1.55 μW/cm^2. Serum exosomes were isolated with ExoQuick solution, and exosomal RNA was prepared using a miRNeasy Micro Kit. The linear range and detection limit of exosomal RNA are 5 fg/mL–50,000 ng/mL and 5 fg/mL, respectively, providing a new direction for quantitative detection of cancer markers.

Nanomaterial-based colorimetric biosensors

Colorimetric biosensors, based on the absorbance and color change of a reaction medium, have aroused extensive attention due to their low readout cost and visual inspection capabilities [113]. The color change can be monitored by the naked eyes or with a photoelectric colorimeter and easily integrated with solid strips, papers, and smartphones. Thus, colorimetric biosensors are a useful, inexpensive, and simple approach, and they have potential applications relevant to daily life. In colorimetric biosensors, the nanomaterials we use most frequently are gold nanoparticles, carbon nanomaterials, and metal oxide nanomaterials.

Gold nanoparticles

Gold nanoparticles (AuNPs) have an ultrahigh extinction coefficient. They are widely used in solution colorimetric analyses. Due to a change in distance between AuNPs, the surface plasmon resonance effect can cause changes in the ultraviolet absorption luminosity of gold nanoparticles or a significant change in solution color [114]. Based on this, Jiang et al. used nucleic acid aptamers and AuNPs to construct biosensors to detect exosome surface protein molecules [43]. The basic principle is that in high salt solution, complexation between the aptamers and AuNPs prevents the AuNPs from aggregating. In the presence of exosomes, the nonspecific and weak binding balance between aptamers and AuNPs will be destroyed. Due to the strong specific binding between the protein on the surface of exosomes and aptamers, aptamers will be rapidly shifted from the AuNPs, resulting in the aggregation and precipitation of the AuNPs, and the solution will change from red to purple to realize visual detection of the protein on the surface of exosomes. This method requires simple equipment and can be observed with the naked eyes, which provides a new research direction for exosome surface protein analysis. Further, Liu et al. established a target-induced proximity ligation trigger recombinase polymerase amplification and transcription-mediated amplification colorimetric assay for highly sensitive and specific detection of nasopharyngeal carcinoma–derived exosomes [44]. The biosensor combines a proximity ligation assay, recombinase polymerase amplification, transcription-mediated amplification, and a gold nanoparticle colorimetric method with a detection limit as low as 100 particles/mL (dispersed in 100 μL PBS). Maiolo et al. established a colorimetric plasma analysis method using gold nanoparticles to determine the purity and titrate of extracellular vesicles [45].

Moreover, gold nanoparticles are typical nanozymes with intrinsic properties mimicking natural enzyme activities [46]. This property allows them to be used in colorimetric exosome biosensors. For example, J. A. Shiddiky used gold-loaded nanoporous ferric oxide nanozymes (AuNPs-Fe_2O_3) to directly isolate and sensitively detect exosomes [47]. First, the surface of AuNPs-Fe_2O_3 was modified with a generic tetraspanin antibody. Then, the above nanoparticles were used to capture the bulk population of exosomes. AuNPs-Fe_2O_3 were detected by an enzyme-linked immunosorbent assay based on their peroxidase-like activity. The linear range and limit of detection were 10^3–10^7 and 10^3 particles/mL, respectively. Di et al. developed a nanozyme-assisted immunosorbent assay for sensitive and rapid multiplex profiling of exosomal proteins [48]. It is based on the installation of 2-nm gold nanoparticles with peroxidase-like properties onto the phospholipid membranes of exosomes. The greatest advantage of this technique is the short test time, without the need for multistep incubation and washing operations.

To make exosome detection more convenient, a paper chip was introduced in a gold nanoparticle–based colorimetric biosensor. Paper chips are well known for their use to qualitatively or quantitatively detect substances through color changes between papers and substances. Since gold nanoparticle–based colorimetric biosensors require a small sample volume, they are convenient to use and more suitable for online monitoring. Recently, lateral flow biosensors have attracted much attention due to their rapid analysis, simplicity, low cost, and high sensitivity and specificity. Oliveira-Rodríguez et al. first established a lateral flow immunochromatographic assay based on gold nanoparticle probes for the detection of exosomes within 15 min [49]. The detection limit, which was determined with a 100-μL sample of exosomes purified from Ma-Mel-86c cell culture supernatants (isolated by the centrifugation method) in HEPES-buffered saline buffer, was as low as 8.5×10^5 particles/mL. Then, they analyzed and compared the detection results of different detection probes (gold nanoparticles, carbon black and magnetic nanoparticles) [50]. Gold nanoparticles exhibited the best sensitivity with a detection limit as low as 3.4×10^9 particles/mL, determined by testing a 100-μL sample of plasma exosomes in HEPES. Yang et al. developed a pH test paper to detect exosomes based on horseradish-peroxidase-mediated promotion of mussel-induced surface engineering and reagent-free functionalization of urease [51]. Urease can hydrolyze urea into ammonia water and carbon dioxide, raising the pH of the solution. Establishing the relationship between pH and exosome concentration can allow convenient measurement of the concentration of exosomes isolated via the ultracentrifugation method using the pH test paper, and the detection limit is as low as 4.5×10^6 particles/mL, determined by testing a 100-μL sample of exosomes secreted by MCF-7 cells in culture medium in PBS.

2D carbon nanomaterials

Carbon nanomaterials have attracted increasing attention in the fabrication of colorimetric biosensors because of their low cost, excellent stability, and bulk preparation capability. Some of them have intrinsic properties mimicking natural enzyme activities. Many scientists are devoted to this research. For example, Wang et al. developed a colorimetric sensor for detecting exosomes by using single-stranded DNA (ssDNA) to enhance the intrinsic peroxidase activity of graphite-phase carbon nitride (g-C_3N_4) nanosheets (Fig. 3) [52]. When the specific aptamer ssDNA of exosome transmembrane protein CD63 is combined with g-C_3N_4, its intrinsic peroxidase activity is enhanced to produce a dark blue product. After exosomes derived from MCF-7 cells and isolated by the ultracentrifugation method are added, ssDNA preferentially binds specifically to exosomes, resulting in a weakened reaction degree and a light blue product. The limit of detection was 1.4×10^9 particles/mL and the detection time was shortened to 30 min. Xia et al. developed an exosome colorimetric sensor based on DNA-capped single-walled carbon nanotubes (s-SWCNTs) and aptamers [53]. An aptamer specific for the exosome transmembrane protein CD63 was adsorbed onto the surface of s-SWCNT and increased the peroxidase activity of s-SWCNTs, which could effectively catalyze H_2O_2-mediated oxidation of 3,3′,5,5′-tetramethylbenzidine 4,4′-bi-2,6-xylidine (TMB) and cause the solution to change from colorless to blue. However, in the presence of exosomes derived from MCF-7 cells (isolated via the ultracentrifugation method and suspended in 100 μL PBS), aptamers could bind to CD63 and desorb from the surface of s-SWCNTs through conformational changes, reverting the s-SWCNTs into its initial state and resulting in a decrease in catalytic activity. The linear range of this method was 1.8×10^9~2.2×10^{10} particles/mL, and the detection limit was 5.2×10^8 particles/mL.

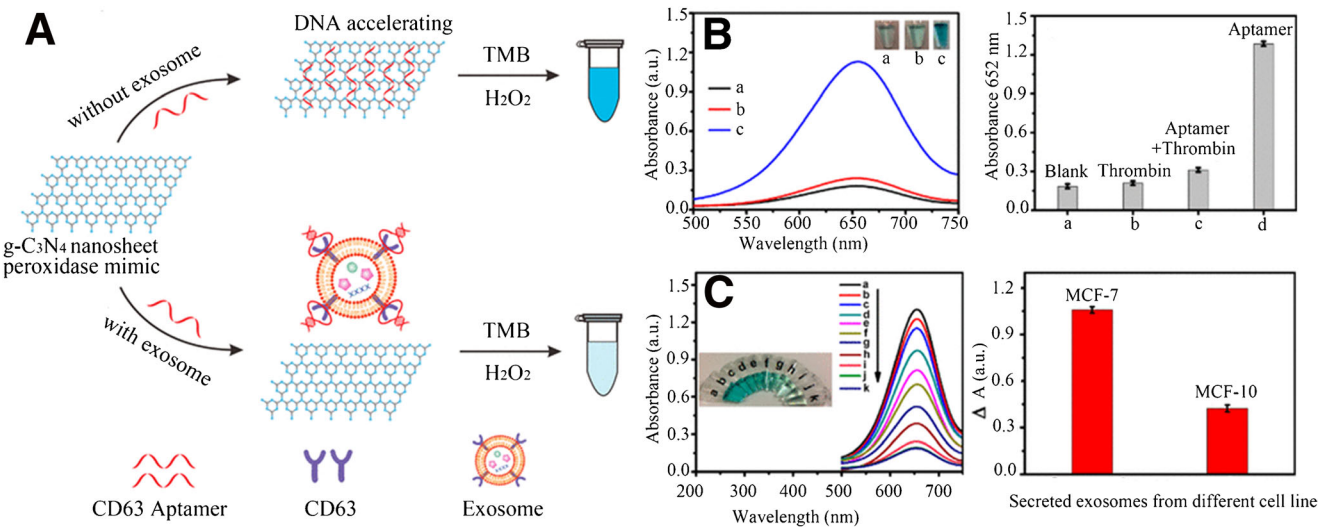

Fig. 3 **A** Schematic illustration of exosome colorimetric biosensors based on DNA aptamers accelerating the intrinsic peroxidase-like activity of g-C_3N_4 NSs, **B** absorption spectra obtained from different catalytic reactions, and **C** research on the linear range and selectivity

Metal oxides

Fe_3O_4 magnetic nanoparticles are particularly interesting in colorimetric biosensors because of their excellent mimic property, low cost, bulk preparation, resistance to pH, and temperature varieties. Chen et al. developed a separation method based on anion exchange, which directly separated exosomes from plasma and cell culture medium through AE magnetic beads within 30 min [54]. Then, for the first time, aptamer-terminated AE magnetic beads were used to detect prostate cancer exosomes in 500 μL plasma in a visualization, unlabeled, quantitative method. The linear range and detection limit of this method were 4.0×10^7–6.0×10^8 particles/mL and 3.6×10^6 particles/mL, respectively. This study provides an effective and practical method for the rapid separation and visual detection of exosomes, which is expected to be used for the early diagnosis of prostate cancer.

Apart from signaling molecules, nanomaterials have also been used as a platform to detect exosomes. Microfluidic chip technology is widely used in exosome separation and the quantification of molecules due to its small size, high integration capability of functional modules, fast analysis speed, and high sensitivity. There are two main methods to capture exosomes with microfluidic chips: one is to modify the substrate with nanomaterials to increase a specific surface area, thus increasing the load of antibodies or aptamers to achieve signal amplification. To make exosome detection more convenient, microfluidics technology and ZnO nanomaterials were introduced to colorimetric exosome biosensors. Chen et al. [55] designed a ZnO nanowire–coated 3D scaffold chip, which was combined with colorimetric methods to detect exosomes. The interconnected micropores of the 3D scaffold induced fluid flow, and the ZnO nanowire array increased the surface area of the immobilized antibody and formed a size exclusion effect, thus enhancing the capture efficiency of exosomes at high flow rates. Finally, horseradish-peroxidase-labeled antibodies were used to detect the captured exosomes, and TMB was added for colorimetric sensing. The linear range was 2.2×10^8 to 2.4×10^{10} particles/mL, and the detection limit was 2.2×10^7 particles/mL (determined by testing a 100-μL sample of exosomes extracted from MCF-7 cells in PBS).

Nanomaterial-based fluorescence biosensors

Fluorescence can be used for qualitative and quantitative analysis by using changes in fluorescence signals, and fluorescence technologies have high sensitivity and good selectivity and are easy to use. Using fluorescence technology and nanomaterials, various nanomaterial-based fluorescence biosensors have been used to determine the concentration of exosomes.

Quantum dots

Quantum dots are commonly used as donors in fluorescence resonance energy transfer systems because of their high fluorescence quantum yield, better light stability, wide excitation wavelength, and narrow and symmetrical size-adjustable emission spectrum, and they are widely used in fluorescence resonance energy transfer research. Xia et al. developed a ratiometric fluorescence bioprobe based on DNA-labeled carbon dots and 5,7-dinitro-2-sulfoacridone coupled with target catalytic signal amplification for detecting MCF-7 cell–derived exosomal microRNA-21 (isolated by the centrifugation method) [56]. When the ratiometric fluorescence bioprobe was assembled, there was a high fluorescence resonance energy transfer efficiency between carbon dots and 5,7-dinitro-2-sulfoacridone. However, in the presence of the target, the fluorescence intensity of the carbon dots and 5,7-dinitro-2-sulfoacridone changed simultaneously as the fluorescent bioprobe decomposed. Due to the ratio of the two fluorescence intensities, the fluorescent bioprobe could offset the environmental fluctuation by calculating the emission intensity ratio of two different wavelengths, which was robust and stable enough to detect exosomal miRNA-21. Using this signal amplification strategy, the detection limit tested with a 100-μL sample of exosomes in PBS was as low as 3.0 fM. More importantly, the strategy could monitor dynamic changes in exosomal microRNA-21; thus, it may become a potential tool to distinguish cancer exosomes from nontumorigenic exosomes. Lateral flow analysis is a widely used method for clinical analysis and evaluation, and possesses the advantages of short analysis, and simple testing and evalution. Fluorescent nanospheres embedded with hundreds of quantum dots are very good reporters for lateral flow assays due to their strong fluorescence intensity, ease of manipulation, good stability, and good biocompatibility [57]. To make the detection of exosomes more convenient and practical, Dong et al. combined fluorescence nanosphere lateral flow analysis and membrane biotinylation strategy to establish a new method to detect exosomes rapidly and sensitively [58]. The detection limit, tested with a 100-μL sample of exosomes obtained by centrifugation in running buffer (0.01 M, pH = 7.4 PBS containing 1% BSA and 1% Tween 20), was as low as 2.0×10^6 particles/mL.

Upconversion nanomaterials

Upconversion nanomaterials are inorganic nanomaterials doped with rare earth ions. Due to their near-infrared excitation property, they can effectively avoid interference from autofluorescence and scattered light from the biological sample itself; further, they can effectively reduce photodamage to the biological sample caused by ultraviolet or visible light excitation. Upconversion nanomaterials are also good

resonant energy donors. Chen et al. established a paper-based aptamer sensor based on the principle of resonant energy transfer between upconversion nanoparticles and gold nanoparticles to detect exosomes isolated by the ultracentrifugation method [59]. When exosomes were present, two parts of the aptamer could bind to CD63 protein on the surface of the exosomes to narrow the distance between the upconversion nanoparticles and gold nanoparticles, thus promoting luminescence quenching. These changes could be monitored with a homemade image system, and the green channel intensity in the obtained color image was extracted using photoshop software to quantify the luminescence. The quenching of upconversion nanoparticles luminescence was linearly related to the concentration of exosomes (1.0×10^7–1.0×10^{11} particles/mL), which could be used to detect exosomes. This method could reach the low detection limit by testing a 10-μL sample of exosomes extracted from human liver cancer HepG2 cells in PBS (1.1×10^6 particles/mL), and it effectively reduced the background signal by using upconversion nanoparticles as luminescent material. Subsequently, they developed a washing-free aptasensor based on rare-earth-doped upconversion nanoparticle donors and tetramethyl rhodamine acceptors for highly sensitive detection of exosomes [60]. The limit of detection was reduced to 8.0×10^4 particles/mL. Similarly, Lyu et al. developed a near-infrared afterglow semiconductor nano-multicomplex for multiple classification and identification of cancer exosomes [61].

Gold nanoparticles

Gold nanoparticles have good surface plasmon resonance properties. This special property endows them with a strong fluorescence quenching ability but does not induce photobleaching. Gold nanoparticles can be used as good fluorescence quenchers in Förster resonance energy transfer (FRET) systems. Zhai et al. developed a new method for in situ detection of plasma exosomal miRNA-1246 based on a gold nanoflare probe for breast cancer diagnosis [62], as shown in Fig. 4. First, the DNA reporter molecule labeled with the fluorescent dye Cy5 was combined with captured DNA, and then, double-stranded DNA was linked to the surface of 13-nm gold nanoparticles to form gold nanoflares. In the resting state, there was resonance energy transfer between the gold nanoparticles and Cy5 that quenched the fluorescence. When the gold nanoflare probes coexisted with breast cancer exosomes, they entered the exosomes, the gold nanoflares dissociated, and the fluorescence signal was recovered. To further improve the detection sensitivity, various types of DNA signal amplification techniques, such as enzyme-free DNA nanomachines [63], hairpin DNA cascade reactions [64], rolling loop amplification reactions [65], and rolling circle amplification reactions [66], have been applied to the detection of fluorescence resonance energy transfer–based detection of exosomes.

2D carbon nanomaterials

Graphene oxide is also a good receptor for fluorescence resonance energy transfer. Compared with other receptors, graphene oxide has high quenching efficiency, which can sufficiently reduce interference from background signals. Due to its large-scale 2D planar structure, it can also serve as a good platform to adsorb specific molecules, such as nonspecific adsorption of single-stranded DNA through π-π stacking between nucleotide bases and carbon planes. Wang et al. developed a deoxyribonuclease I enzyme-assisted fluorescent signal amplification method based on graphene oxide-DNA aptamer interactions to detect colorectal cancer exosomes [67]. First, the fluorescent dye–labeled aptamer was combined with graphene oxide to quench the fluorescence of the dye. In the presence of exosomes, dye-labeled aptamers detached from the graphene oxide due to the stronger specific interaction between aptamers and exosomes. Under the action of DNase I enzymes, the aptamers on the surface of exosomes

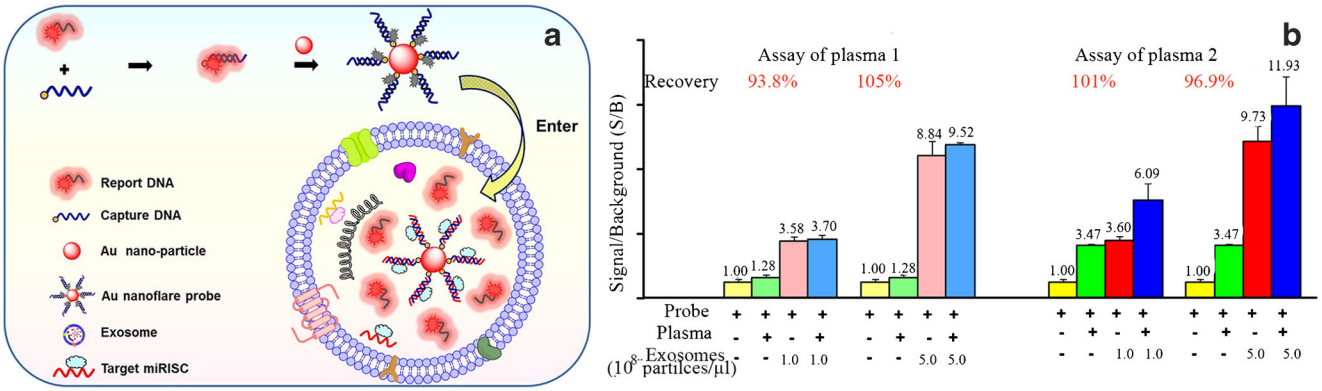

Fig. 4 **a** Schematic illustration of in situ detection of plasma exosomal microRNA-1246 for breast cancer diagnostics using an Au nanoflare probe. **b** The miR-1246 probe detects exosomes spiked into human plasma with a good recovery rate

disintegrated, dyes were released into the solution, and the fluorescence was restored. There was a linear relationship between the degree of fluorescence recovery and the number of exosomes. The detection limit of this method, determined by testing 1000 μL of human colorectal cancer cell line SW480 exosomes in PBS, was 2.1×10^7 particles/mL. Zhou et al. developed a new sensitive detection method for early markers of Alzheimer's disease in exosomes based on signal amplification technology [68]. Jin et al. established an exosome-oriented, aptamer graphene oxide nanoprobe-based profiling assay to phenotype surface proteins and quantify cancerous exosomes in a facile mix-and-detect format [69]. To make exosome detection more convenient, microfluidics technology was introduced to fluorescence exosome biosensors. Zhang et al. [70] developed a microfluidic exosome analysis platform based on a novel graphene oxide/polydopamine nanointerface, which was equipped with a series of Y-shaped microcolumns, increasing the surface area of capture antibody fixation and greatly improving the efficiency of exosome capture. The efficiency of capture, while effectively inhibiting nonspecific exosome adsorption, could better detect exosomes with a detection limit of 5.0×10^4 particles/mL (determined by testing a 20-μL sample of exosomes purified from COLO-1 cell culture supernatants in PBS).

MXenes is a general term for 2D transition metal carbide and carbonitride materials, which usually have hydrophilicity, metal conductivity, and a large specific surface area. Because their surface is rich in oxygen and hydroxyl groups, they can connect biomolecules through hydrogen bonds, van der Waals forces, electrostatic action, and coordination action. In addition, MXene nanosheets have the property of autofluorescence. The inherent fluorescence quenching ability, unique self-fluorescence characteristics, and surface characteristics of transition metal ions on MXenes make them suitable for biosensing research. Zhang et al. constructed a self-ratiometric fluorescence resonance energy transfer platform based on Ti_3C_2 MXenes to detect exosomes with high sensitivity [71]. A Cy3-CD63 aptamer can be selectively adsorbed onto Ti_3C_2 MXene nanosheets through hydrogen bonds and metal chelation interactions between the aptamer and MXene, and due to the FRET between Cy3 and MXene, fluorescence signals from the Cy3-CD63 aptamer are rapidly quenched. After the addition of exosomes, the fluorescence of Cy3 is greatly recovered, and exosomes can specifically bind to aptamers and are released from the surface of Ti_3C_2 MXenes due to the high affinity of aptamers and CD63 protein on the surface of exosomes. Meanwhile, the autofluorescence signal of MXenes shows little change during the entire process and thus can be used as a standard reference. Based on the FRET biosensor platform opened from the standard, the detection limit of cell-derived exosomes (isolated by the centrifugation method) was 1.4×10^6 particles/mL, which is more than 1000 times lower than that of the conventional ELISA method (determined by testing a 300-μL sample).

Metal oxides

Metal oxides with nanostructures have also attracted extensive interest as fluorescence exosome biosensors. On the one hand, they have been used as a platform to capture exosomes. For example, Chen et al. designed a sandwich-type fluorescence biosensor for the determination of tumor-related exosomes based on magnetic Fe_3O_4 nanoparticle capture and horseradish peroxidase catalysis [72]. First, magnetic nanoparticles were modified with CD63 antibody, and then, the antibody was used as the substrate to capture exosomes. Subsequently, the tumor-related exosomes were captured by biotinylated epithelial cell adhesion molecule antibody. Exosomes from the plasma of patients with hepatocellular carcinoma were obtained by ultracentrifugation to prepare a 200 μL dispersion, and the linear range was from 576 to 5.76×10^7 particles/mL, with a detection limit of 200 particles/mL. Similarly, Shi et al. developed a fluorometric assay for the determination of exosomes based on hybridization chain reaction and magnetic nanoparticles [73]. The linear range was from 1000 to 10^7 particles/mL, with a detection limit of 100 particles/mL.

Recently, copper oxide nanoparticles, a metal oxide nanomaterial, were utilized to develop a new method for exosome quantification based on a copper-mediated signal amplification strategy [74]. First, cholesterol-modified magnetic beads captured exosomes through hydrophobic interactions between cholesterol and lipid membranes. Then, the captured exosomes were combined with the aptamer-modified copper oxide nanoparticles to form a sandwich complex. The copper oxide nanoparticles were dissolved by acid to form divalent copper ions. Bivalent copper ions could be reduced to fluorescent copper nanoparticles by sodium ascorbate in the presence of poly(thymine). Quantitative detection of exosomes (isolated using an exoEasy Maxi Kit) could be achieved by observing changes in the fluorescence signals of the copper nanoparticles. The linear correlation range and detection limit determined by testing a 310-μL sample of exosomes (in MOPS buffer) secreted by HepG2 cells in culture medium were 7.5×10^7–1.5×10^{10} particles/mL and 7.5×10^7 particles/mL, respectively.

Other nanomaterials

Moreover, nanoscale lipid vesicles and DNA nanotechnology present a new approach to developing new types of nanomaterials with high quality for exosome detection. Wang et al. proposed synthesizing a new exosome-zirconium-liposome sandwich structure based on zirconium-phosphate coordination for the detection of exosomes [75]. Liposomes bound to exosomes release fluorescent calcium protein in the presence

of Triton X-100. There was a linear relationship between the amount of calcium and the number of exosomes, with a detection limit as low as 7.6×10^6 particles/mL. The method has the advantages of low cost, no label modification, simplicity, and high efficiency. He et al. established a new method for the detection of individual exosomes in plasma samples based on DNA nanodevices [76].

Nanomaterial-based chemiluminescence biosensors

Chemiluminescence is a powerful and important optical approach based on electromagnetic radiation emitted by an exothermic chemical reaction without using external light sources or optics [115, 116]. Due to its high sensitivity, wide linear dynamic range, simple instrumentation, fast analysis, and low background, it is widely used in the field of biosensors [117]. However, practical application of chemiluminescence-based biosensors for the detection of exosomal biomarkers is limited by the small chemiluminescence reaction system and poor selectivity. Therefore, scientists have used metal oxide nanomaterials, including iron oxide and CuS nanoparticles, to overcome these shortcomings. Wang et al. developed a superparamagnetic iron oxide particle–based chemiluminescent immunoassay for rapid and quantitative analysis of exosomes from biofluids [77]. Anti-CD63 antibody–conjugated superparamagnetic iron oxide particles (SIOPs) and acridinium ester–labeled anti-CD9 antibodies formed a double-antibody sandwich system. Exosomes from pancreatic tumor cell line culture medium were isolated by ultracentrifugation. The linear range determined by a 1 μL exosome sample was 2.9×10^8 to 2.8×10^{11} particles/mL, with a detection limit of 2.6×10^8 particles/mL. Jiang et al. established a novel method for the rapid enrichment and detection of extracellular vesicles based on CuS-enclosed microgels [78]. A Sorvall ST 16 centrifuge and an Optima XPN-80 ultracentrifuge were used to isolate extracellular vesicles. A detection limit of 10^4 extracellular vesicle particles/mL was achieved with these microgels by detecting extracellular vesicles in human serum and cell culture medium, with a dynamic range up to 10^8 particles/mL.

Nanomaterial-based electrochemiluminescence biosensors

Electrochemiluminescence (ECL) is a combination of chemiluminescence and electrochemistry. ECL refers to the luminescence phenomenon when an electrochemical reaction is caused by the application of a certain voltage to generate a new substance on the electrode surface and further reaction between electrobiomasses or between electrobiomasses and other substances. With the advantages of high sensitivity, wide dynamic range, strong anti-interference capability, good reproducibility, good selectivity, simple equipment, and simple operation, ECL is widely used in the field of biosensing. With the rapid development of nanomaterials, scientists have paid much attention to nanomaterial-based ECL biosensors.

Carbon-based nanomaterials

Ti_3C_2 MXene nanoplates are useful ECL nanoprobes due to their large specific surface area, satisfactory electrical conductivity, and catalytic activity. Zhang et al. developed a 2D Ti_3C_2 MXene nanosheet catalytic electrochemiluminescence biosensor with high efficiency for the detection of exosomes [79]. First, the electrode surface was modified with an aptamer that recognized the EpCAM protein, and the exosomes were efficiently captured on the electrode surface. Then, the ECL nanoprobe recognized 100 μL of MCF-7 exosomes (isolated by centrifugation) and significantly enhanced the ECL signals of luminol. Based on this strategy, a highly sensitive ECL biosensor for MCF-7 exosome detection was obtained. The detection limit is 1.3×10^5 particles/mL, which is over 100 times lower than that of the traditional ELISA method. This strategy provides a feasible, sensitive, and reliable tool for exosome detection in clinical diagnosis of exosomes. To improve the ECL signal, a gold-nanoparticle-decorated Ti_3C_2 MXene hybrid with aptamer modification was introduced [80]. The detection limit was 3.0×10^4 particles/mL for exosomes derived from the HeLa cell line, and the linear range was from 10^5 to 10^8 particles/mL.

However, the high background ECL signal from the blank samples may cause a false-positive result and limit sensitivity, especially for real samples in the presence of abundant interfering factors. To solve this problem, Liu et al. developed an ECL exosome biosensor based on a multivalent interface and a g-C_3N_4-coated liquid metal nanoprobe [81]. Exosomes derived from HeLa cells were isolated by centrifugation and suspended in 200 μL PBS. The limit of detection was 31 particles/μL, and the linear range was over 3 orders of magnitude. Exosomes in serum, urine, and blood samples were also applied, verifying the potential application of the nanoprobe in clinical diagnosis. Silica nanoparticles were also used to package abundant $Ru(bpy)_3^{2+}$ molecules to strengthen the ECL intensity [82]. Exosomes derived from HepG2 cells were isolated by ultracentrifugation and suspended in 100 μL PBS. The detection linear range was from 2.0×10^5 to 7.5×10^7 particles/mL, with a detection limit of 6.0×10^4 particles/mL.

Quantum dots

Quantum dots exhibit excellent luminescence with adjustable wavelengths from ultraviolet light to near-infrared light [118]. This makes them important nanoprobes in ECL biosensors. Shen et al. developed a novel ECL aptasensor for the detection

of exosomes from MCF-7 cells based on mercaptopropionic acid–modified Eu^{3+}-doped CdS nanocrystal ECL emitters [83]. Exosomes were isolated by the centrifugation method and suspended in 50 μL PBS. The limit of detection was 7.4×10^4 particles/mL, and the linear range was 3.4×10^8 to 1.7×10^{11} particles/mL. Exosomes in serum were also applied, verifying the potential application of this sensor in clinical diagnosis. Dai et al. developed an ECL and photothermal dual-mode biosensor for the determination of MCF-7 cell–derived exosomes based on black phosphorus quantum dot–functionalized MXenes as a signal amplifier [84]. The limit of detection was 3.7×10^4 particles/mL, and the liner range was from 1.1×10^5 to 1.1×10^{10} particles/mL.

Nanomaterial-based surface plasmon resonance biosensor

Surface plasmon resonance (SPR) is an optical phenomenon that is used to track real-time interactions between biological molecules in their natural state. Its detection principle is as follows: first, a bioreceptor is bonded to the surface of the biosensor, and then, a solution containing another biomolecule capable of interacting with the bioreceptor is injected and flows across the biosensor surface. The combination of biomolecules leads to an increase in the surface quality of the biosensor, resulting in an increase in the refractive index in the same proportion, and changes in the biomolecular reactions are observed. Generally, SPR can induce signal changes within 200 nm from the gold surface, and the size of exosomes is typically approximately 100 nm. Therefore, the use of SPR to quantify exosomes combines the advantages of SPR and exosomes at the same time. The high molecular weight of exosomes makes this method highly sensitive. In addition, the method has the advantages of no preprocessing, real-time analysis, and no sample labeling [119].

Gold nanomaterials

Gold nanomaterials are commonly used as a signal amplification probe in the SPR method. Im et al. first constructed a quantitative analysis method (nPLEX) of nanoplasma exosomes based on gold nanopores [85]. In this method, a periodic nanoporous array was designed on the gold membrane. Each array was immobilized with antibodies specific to exosomal proteins. When exosomes and nPLEX sensors specifically bound through antigen-antibody interaction, the spectrum peak was shifted, and the displacement size was proportional to the quality of exosomes so that the exosomes could be detected. Because the exosome itself can cause displacement changes, it can be used for unlabeled measurement. Duraichelvan et al. established a label-free local surface plasmon resonance method for exosome detection based on gold nanoparticles [86]. Subsequently, Giuseppe established a new method to obtain exosomes from individuals with multiple myeloma or monoclonal gammopathy and healthy individuals using surface plasmon resonance sensing of gold nanoparticles [87]. Park et al. first constructed an SPR sensor using gold nanoparticles for the quantitative analysis of intracapsular exosomal proteins [88]. Exosomes and microvesicles share many characteristics, but it is important to distinguish between the two types of extracellular vesicles. Thakur et al. developed a local surface plasmon resonance biosensor with self-assembled gold nanoislands that can be used to detect and distinguish microcapsules and exosomes among extracellular vesicles separated from A-549 cells, SH-SY5Y cells, serum, and urine [89]. The linear range of the sensor was 0.194~100 μg/mL, and the detection limit was 0.194 μg/mL (determined by testing a 100-μL sample of exosomes extracted from human lung cancer A549 cells in PBS). Using this method, exosomes and microbubbles secreted by tumor cells could be directly detected without functionalization with antibodies. Wang et al. established an SPR sensor for exosome detection based on a double gold nanoparticle signal amplification strategy [90]. The detection limit of this method was as low as 5000 particles/mL, and the LOD was 10^4 times lower than that of commercial ELISA (exosomes extracted from MCF-7 breast cancer cells in culture medium). However, the method to analyze the different exosomes was limited. To solve this problem, Ding et al. developed a high-sensitivity and multiplex biosensing assay of NSCLC-derived exosomes via different recognition sites based on an SPRi array and gold nanoparticles [91]. In this way, exosomes derived from normal lung and NSCLC cells could be effectively distinguished through precise identification of the exosomal protein pattern.

Titanium nitride nanomaterials

Titanium nitride (TiN) is an excellent alternative plasmonic supporting material with adjustable and steady electroconductibility and tunable plasmonic properties in the near-infrared visible spectrum. Qiu et al. developed a biotinylated antibody-functionalized TiN plasmonic biosensor for the determination of glioma-derived exosomes isolated from a human glioma cell line [92]. First, a TiN nanofilm was synthesized by the magnetron sputtering method and functionalized with a biotinylated anti-EGFRvIII antibody based on the high-affinity biotin-TiN interaction. Then, the prepared substrate was employed as a reporter in plasmonic biosensing to supply good sensitivity and selectivity towards GM-derived exosomes. The detection limits for CD63 and epidermal growth factor receptor variant-III were 4.29×10^{-3} μg/mL and 2.75×10^{-3} μg/mL, respectively. Compared with conventional gold film biosensors, this SPR biosensor exhibited better sensitivity and higher stability. Moreover, it has the prospect of detection in real biological fluids.

Magnetic nanomaterials

Exosomes are nanosized (30–100 nm in diameter) extracellular vesicles secreted by mammalian cells. Their diffusion speed is very slow, which limits the yield in the collection of exosomes on a sensor surface. To solve this problem, Reiner et al. developed a magnetic nanoparticle–enhanced surface plasmon resonance biosensor for the determination of extracellular vesicles by taking advantage of the highly efficient accumulation of exosomes on the sensor surface [93]. The surface of the gold-coated sensor chips was modified with antibody. Exosomes and streptavidin-coated MNPs were captured one by one. By coupling MNPs with a magnetic field gradient, efficient accumulation of exosomes on the sensor surface was achieved. The enhancement signal was obtained by a combined effect of increased speed of binding kinetics and enhanced surface mass density. The detection limit of exosomes secreted by mesenchymal stem cells reached as low as 0.76 μg/mL.

Nanomaterial-based surface-enhanced Raman spectroscopy biosensors

Raman spectroscopy is a spectral analysis method that analyzes the vibration mode of a sample by measuring the inelastic scattering effect caused by a radiation laser [120]. Because of its high sensitivity, nondestructive, and invasive properties, Raman spectroscopy is suitable for use in fields such as biosensors [121–123]. However, since Raman scattering is uncommon, the signal intensity is very low, and it is difficult to distinguish. To overcome this problem, Raman spectroscopy techniques have been developed to enhance the electric field by surface treatment [124, 125]. SERS is a type of molecular vibration spectrum that can enhance Raman scattering signals by molecules adsorbed on rough metal surfaces or nanostructures, such as plasma magnetic silicon dioxide nanotubes. In recent years, many surface-enhanced Raman techniques combined with metal nanomaterials have been applied to the detection of exosomes due to the strong electric field enhancement position on the surface of metal nanostructures, i.e., SERS hotspots.

Metal nanomaterials have become the most widely used SERS substrates due to their small size, large specific surface area, and unique optical, electrical, and physicochemical properties. Various metal nanomaterials have been applied to the detection of exosomes. Stremersch et al. first used gold nanoparticles as SERS signal probes to establish a new method for exosome characterization [94]. Park et al. attached exosomes directly to gold-nanoparticle-coated glass slides and analyzed the entire SERS spectrum using principal component analysis (PCA) to distinguish exosomes secreted by lung cancer cells from those secreted by normal cells [95]. The sensitivity and specificity of this method were 95.3% and 97.3%, respectively. By combining SERS and PCA, the method can be used for real-time diagnosis and identification of cancer with high sensitivity and without the need for labeling. Lee et al. reported a study of exosomes in solution using a 3D nanosilver bowl platform [96]. Combined with SERS, this nanobowl-shaped plasma structure is capable of capturing and analyzing exosomes. Then, with silver nanoparticles as signal probes, they used lectin-specific peptide ligands to selectively capture exosomes and perform the SERS method [97]. Kwizera et al. developed a miniaturized device for quantitative detection of exosomes using a gold nanorod SERS signal probe and low-cost 3D printing technology [98]. Ertsgaard et al. integrated subvoltaic medium electrophoresis capture and gold nanoparticles to develop a nanogap platform for real-time imaging of exosomes [99]. Recently, Lee et al. used a uniform plasma gold nanoclustered matrix to obtain multiple hotspot signals to establish a new method for quantitative detection of exosomal miRNAs [100]. The sensor, which has a very low detection limit and a wide dynamic range (1 aM to 100 nM, tested with 200 samples of exosomes extracted from cells in culture medium) and does not require any amplification process, can differentiate breast cancer subtypes by observing the expression patterns of microRNAs in the exosomes of breast cancer cell lines. Simultaneous detection of multiple components is also an important research direction in biological analysis. Wang et al. developed a new method for simultaneous detection of tumor-derived exosomes based on magnetic substrates and gold nanoparticle SERS probes [101]. In exosomes, the capture substrate was mixed with three SERS probes to form an aptamer-immune complex, and other nonspecific SERS probes remained in suspension. The method has been successfully applied to the detection of exosomes in real blood samples.

Combinations of Au@Ag or Ag@Au core-shell colloids have the properties of two metals, thus widening the available excitation interval and obtaining adjustable SPR characteristics, outstanding enhancement, and stability. Among various SERS substrates, Au nanostructures are easy to prepare and have good stability, while Ag nanostructures exhibit higher SERS enhancement, but it is difficult to control their size and shape. Therefore, by combining their advantages, many core-shell structures can be designed to achieve strong SERS enhancements. Using SERS, Zong et al. constructed a SERS nanoprobe and magnetic nanobead-based platform for detecting tumor-derived exosomes [102]. As shown in Fig. 5, this method takes advantage of the immunoaffinity between different proteins carried on exosomes and corresponding antibodies. The surface of SERS nanoprobes with magnetic nanobeads and gold core-silver shell nanorods were modified with two types of specific antibodies to identify two different proteins on exosomes. When exosomes are added, an immune complex of sandwich structure could form among the three components. In the presence of magnets, immune complexes

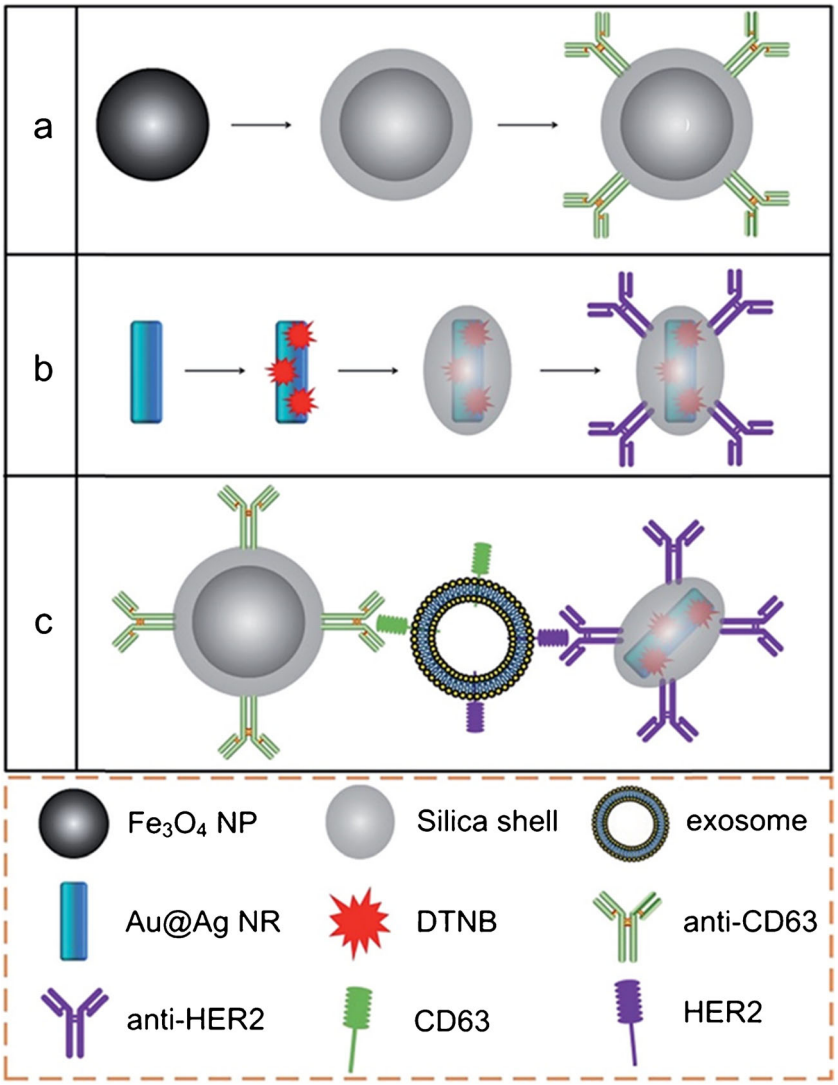

Fig. 5 Schematic illustration of the SERS-based exosome detection method

could precipitate, and SERS nanoprobes were able to detect SERS signals in the precipitate. However, in the absence of target exosomes, since immune complexes could not be formed, only magnetic nanoparticles were contained in the precipitate, and SERS signals cannot be detected. The detection limit is 1.2×10^6 particle/20 mL in deionized water medium, and the detection time is 0.5 h, which is much lower than the time required for traditional enzyme-linked immunosorbent assays. To further improve sensitivity, Ma et al. developed a new SERS sensing method for quantitative detection of human blood exosomal miRNA using Au@R6G@AgAu nanoparticles as signal probes and combining silicon magnetic bead separation and chain-specific nuclease (DSN)–assisted signal amplification strategies [103]. With a detection limit as low as 5 fM and a sample size of only 5 μL, the method has potential clinical application prospects. Pang et al. developed a SERS biosensor that uses Au@Ag nanorods@DTNB as a SERS signal probe and combines Fe_3O_4@Ag nanomaterials and a chain-specific nuclease (DSN)–assisted signal amplification strategy for quantitative detection of microRNA-10b in exosomes and residual plasma [104]. Dual SERS enhancements and recirculation signal amplification made the detection limit as low as 1 aM. The method could directly and quantitatively detect exosomal microRNA in pancreatic ductal adenocarcinoma, chronic pancreatitis, and healthy control group plasma samples. Li et al. also constructed a SERS immunosensor based on polydopamine-functionalized Au@Ag nanoparticles for the classification of pancreatic cancer [105]. Fraire et al. developed an Au@Ag nanoparticle SERS biosensor for label-free identification of individual exosome-like vesicles [106]. Pang et al. developed a SERS immunoassay based on Fe_3O_4@TiO_2 isolation and Au@Ag nanoparticle SERS tags [107]. Tian et al. constructed a biosensor using gold nanosatellite@gold nanoshell as a SERS nanoprobe to realize sensitive detection of exosomes [108]. This method had a

linear range of 4.0×10^4–4×10^{10} particles/mL and a detection limit of 2.7×10^4 particles/mL (determined by testing a 200-μL sample of exosomes purified from HepG2 cell culture supernatants in PBST).

Other nanomaterial-based biosensors

In addition to the abovementioned biosensors, other detection biosensors are also used in the detection of exosomes, such as nanomechanical [126] and thermal biosensors [127]. Etayash et al. reported a nanomechanical system based on gold nanoparticles for simultaneous detection of exosome surface antigens [126]. The novel trace exosome detection chip used a nanocantilever to fix antibodies in a specific position and used antibodies to capture and enrich trace exosomes in the sample for analysis. The method had high specificity and sensitivity. However, the detection of protein polysaccharides and phosphatidylinositol proteoglycan-1 on the surface of target cells was limited (200 particles/mL, 0.1 pg/mL, determined by testing a 1-mL sample of exosomes extracted from cells in sterile buffer). Smartphone-based thermal readers have the advantages of high sensitivity and expedient portability and thus have received much attention. Cheng et al. developed an Au@Pd nanopopcorn– and aptamer nanoflower–assisted lateral flow strip for thermal detection of exosomes [127]. The limit of detection (LOD) was 1.4×10^7 particles/mL, which indicated a 71-fold improvement in analytical performance.

Summary and prospect

The early diagnosis of cancer has become an urgent clinical issue. The development of noninvasive fluid biopsy technologies has provided technical support for early diagnosis, accurate treatment, and prognosis evaluation of cancer. Exosomes in bodily fluids have broad application prospects as potential biomarkers in liquid biopsy detection. Since the development of nanotechnology, there has been a new trend to apply nanomaterials to the traditional analysis of exosomes. In this paper, the application progress in nanomaterial-based biosensors in exosome detection is summarized from the aspects of electrochemical biosensors, photoelectrochemical biosensors, colorimetric biosensors, fluorescence biosensors, chemiluminescence biosensors, electrochemiluminescence biosensors, surface plasmon resonance biosensors, surface-enhanced Raman spectroscopy biosensors, and other methods. However, research on exosome detection technology is still in its initial stage, and there are many pressing problems, such as how to improve the stability for exosomes analysis; how to identify exosomes secreted from almost all body cells; how to reduce the operation steps, shorten the detection time, and reduce the cost; and how to design robust and point-of-care equipment with simple operation processes.

In the future, there will be higher requirements for the precision of exosome quantitative devices and equipment. Combining exosome separation platforms with accurate exosome quantification technology will become the new trend, which will aid in the study of the biological significance of exosomes and will produce revolutionary optimization of clinical diagnosis and treatment using exosomes. In recent years, the number of potential biomarkers for use in early diagnosis and treatment of cancer may have rapidly increased, but their clinical applications are still limited. Reliable identification and verification of biomarkers contained in exosomes as new indicators of cancer diagnosis, treatment, and prognosis are still of vital importance. In the future, the stability, selectivity, sensitivity, and throughput capacity of exosome detection will be improved in the following aspects to make detection more suitable for clinical application:

(1) Single molecule detection: The current detection system is limited to the average signal of exosomes. In the future, the sensitivity of the system will be improved, and single exosome analysis will be carried out to better understand exosome biology and the role of exosomes in intercellular communication and tumor heterogeneity.
(2) The search for exosome-targeting molecules with better specificity.
(3) The development of unmarked methods to simplify the workflow and reduce the occupied space of instruments.
(4) The development of low-cost and high-throughput separation sensor chips.
(5) The expansion of the signal transduction and amplification approach of the sensor.
(6) The development of robust, user-friendly, and point-of-care instruments.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA-Cancer J Clin. 2019;69:7–34.
2. Thery C, Witwer KW, Aikawa E, Alcaraz MJ, et al. Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines. J Extracell Vesices. 2018;7:1535750.

3. Jalalian SH, Ramezani M, Jalalian SA, Abnous K, Taghdisi S. Exosomes, new biomarkers in early cancer detection. Anal Biochem. 2019;571:1–13.
4. Trams EG, Lauter CJ, Salem JN, Heine U. Exfoliation of membrane ecto-enzymes in the form of micro-vesicles. BBA-Biomembr. 1981;645:63–70.
5. Miller IV, Grunewald TG. Tumour-derived exosomes: tiny envelops for big stories. Biol Cell. 2015;107:287–305.
6. Mathivanan S, Fahner CJ, Reid GE, Simpson RJ. Exocarta 2012: database of exosomal proteins, RNA and lipids. Nucleic Acids Res. 2012;40:D1241–4.
7. Madison MN, Okeoma CM. Exosomes: implications in HIV-1 pathogenesis. Viruses. 2015;7:4093–118.
8. Tang MK, Wong AS. Exosomes: emerging biomarkers and targets for ovarian cancer. Cancer Lett. 2015;367:26–33.
9. Pan J, Ding M, Xu K, Yangm C, Mao LJ. Exosomes in diagnosis and therapy of prostate cancer. Oncotarget. 2017;8:97693–700.
10. Masuyk AI, Masyuk TV, Larusso NF. Exosomes in the pathogenesis, diagnostics and therapeutics of liver diseases. J Hepatol. 2013;59:612–5.
11. Kawikova I, Askenase PW. Diagnostic and therapeutic potentials of exosomes in CNS diseases. Brain Res. 2015;1617:63–71.
12. Zheng X, Chen F, Zhang J, Zhang Q, Lin J. Exosome analysis: a promising biomarker system with special attention to saliva. J Membr Biol. 2014;247:1129–36.
13. Kowal EJK, Ter-ovanesyan D, Regev A, Church GM. Extracellular vesicle isolation and analysis by Western blotting. Methods Mol Biol. 2017;1660:143–52.
14. Lee J, Kim H, Heo Y, Yoo YK, Han SI, Kim C, et al. Enhanced paper-based ELISA for simultaneous EVs/exosome isolation and detection using streptavidin agarose-based immobilization. Analyst. 2020;145:157–64.
15. Brown BA, Zeng XY, Todd AR, Barnes LF, Winstone JMA, Trinidad JC, et al. Charge detection mass spectrometry measurements of exosomes and other extracellular particles enriched from bovine milk. Anal Chem. 2020;92:3285–92.
16. Dragovic RA, Gardiner C, Brooks AS. Sizing and phenotyping of cellular vesicles using nanoparticle tracking analysis. Nanomedicine. 2011;7:780–8.
17. Su J, Song S. A review of the quantitative detection and diagnostic and therapeutic applications of exosomes. J Radiat Res Radiat Process. 2017;35:030101–10.
18. Pol E, Coumans FAW, Grootemaat AE, Gardiner C, Sarqent IL, Harrison P, et al. Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. J Thromb Haemost. 2014;12:1182–92.
19. Baptista FR, Belhout SA, Giordani S, Quinn SJ. Recent developments in carbon nanomaterial sensors. Chem Soc Rev. 2015;44:4433–53.
20. Zhu C, Li L, Wang Z, Irfan M, Qu F. Recent advances of aptasensors for exosomes detection. Biosens Bioelectron. 2020;160:112213.
21. Shao B, Xiao Z. Recent achievements in exosomal biomarkers detection by nanomaterials-based optical biosensors-a review. Anal Chim Acta. 2020;1114:74–84.
22. Xu L, Shoaie N, Jahanpeyma F, Zhao J, Azimzadeh M, Jamal KTA. Optical, electrochemical and electrical (nano)biosensors for the detection of exosomes: a comprehensive overview. Biosens Bioelectron. 2020;161:112222.
23. Fu Z, Lu Y, Lai JJ. Recent advances in biosensors for nucleic acid and exosome detection. Chonnam Med J. 2019;55:86–98.
24. Xu H, Ye BC. Advances in biosensing technologies for analysis of cancer-derived exosomes. Trends Anal Chem. 2020;123:115773.
25. Im H, Lee K, Weissleder R, Lee H, Castro CM. Novel nanosensing technologies for exosome detection and profiling. Lab Chip. 2017;17:2892–8.
26. Cheng N, Du D, Wang X, Liu D, Xu W, Luo Y, et al. Recent advances in biosensors for detection cancer-derived exosomes. Trends Biotechnol. 2019;37:1236–54.
27. Wei F, Yang J, Wong DTW. Detection of exosomal biomarker by electric field-induced release and measurement (EFIRM). Biosens Bioelectron. 2013;44:115–21.
28. Zhou Q, Rahimian A, Son K, Shin DS, Patel T, Revzin A. Development of an aptasensor for electrochemical detection of exosomes. Methods. 2016;97:88–93.
29. Wang S, Zhang L, Wan S, Cansiz S, Cui C, Liu Y, et al. Aptasensor with expanded nucleotide using DNA nanotetrahedra for electrochemical detection of cancerous exosomes. ACS Nano. 2017;11:3943–9.
30. Miao P, Tang Y. Multipedal DNA walker for amplified detection of tumor exosomes. Chem Commun. 2020;56:4982–5.
31. Zhou YG, Mohamadi RM, Poudineh M, Kermanshah L, Ahmed S, Safaei TS, et al. Interrogating circulating microsomes and exosomes using metal nanoparticles. Small. 2016;12:727–32.
32. Boriachek K, Islam MN, Gopalan V, Lam AK, Nguyen NT, Shiddiky MJA. Quantum dot-based sensitive detection of disease specific exosome in serum. Analyst. 2017;142:2211–9.
33. Boriachek K, Umer M, Islam MN, Gopalan V, Lam AK, Nguyen NT, et al. An amplification-free electrochemical detection of exosomal miRNA-21 in serum samples. Analyst. 2018;143:1662–9.
34. Lim J, Choi M, Lee HJ, Han JY, Cho Y. A novel multifunctional nanowire platform for highly efficient isolation and analysis of circulating tumor-specific markers. Front Chem. 2019;6:664–71.
35. Sun Z, Wang L, Wu S, Pan Y, Dong Y, Zhu S, et al. An electrochemical biosensor designed by using Zr-based metal-organic frameworks for the detection of glioblastoma-derived exosomes with practical application. Anal Chem. 2020;92:3819–26.
36. Sun Y, Jin H, Jiang X, Gui R. Assembly of black phosphorous nanosheets and MOF to form functional hybrid thin-film for precise protein capture, dual-signal and intrinsic self-calibration sensing of specific cancer-derived exosomes. Anal Chem. 2020;92:2866–75.
37. Cao Y, Li LL, Han B, Wang Y, Dai YH, Zhao J. A catalytic molecule machine-driven biosensing method for amplified electrochemical detection of exosomes. Biosens Bioelectron. 2019;141:111397–402.
38. Huang RR, He L, Xia YY, Xu PP, Liu C, Xie H, et al. A sensitive aptasensor based on a hemin/G-quadruplex-assisted signal amplification strategy for electrochemical detection of gastric cancer exosomes. Small. 2019;15:1900735–40.
39. Dong HL, Chen HF, Jiang JQ, Zhang H, Cai CX, Shen QM. Highly sensitive electrochemical detection of tumor exosomes based on aptamer recognition-induced multi-DNA release and cyclic enzymatic amplification. Anal Chem. 2018;90:4507–13.
40. Zhang J, Wang LL, Hou MF, Xia YK, He WH, Yan A, et al. A ratiometric electrochemical biosensor for the exosomal microRNAs detection based on bipedal DNA walkers propelled by locked nucleic acid modified toehold mediate strand displacement reaction. Biosens Bioelectron. 2018;102:33–40.
41. Zhao L, Sun R, He P, Zhang X. Ultrasensitive detection of exosomes by target-triggered three-dimensional DNA walking machine and exonuclease III-assisted electrochemical ratiometric biosensing. Anal Chem. 2019;91:14773–9.
42. Pang XH, Zhang X, Gao KK, Wan S, Cui C, Li L, et al. Visible light-driven self-powered device based on a straddling nano-he88terojunction and bio-application for the quantitation of exosomal RNA. ACS Nano. 2019;13:1817–27.

43. Jiang Y, Shi M, Liu Y, Wan S, Cui C, Zhang L, et al. Aptamer/AuNP biosensor for colorimetric profiling of exosomal proteins. Angew Chem Int Ed. 2017;129:11916–20.
44. Liu WL, Li JP, Wu YX, Xing S, Lai YZ, Zhang G. Target-induced proximity ligation triggers recombinase polymerase amplification and transcription-mediated amplification to detect tumor-derived exosomes in nasopharyngeal carcinoma with high sensitivity. Biosens Bioelectron. 2018;102:204–10.
45. Maiolo D, Paolini L, Noto GD, Zendrini A, Berti D, Bergese P, et al. Colorimetric nanoplasmonic assay to determine purity and titrate extracellular vesicles. Anal Chem. 2015;87:4168–76.
46. Wang XY, Hu YH, Wu H. Nanozymes in bionanotechnology: from sensing to therapeutics and beyond. Inorg Chem Front. 2016;3:41–60.
47. Boriachek K, Masud MK, Palma C, Phan HP, Yamauchi Y, Hossain MSS, et al. Avoiding pre-isolation step in exosome analysis: direct isolation and sensitive detection of exosomes using gold-loaded nanoporous ferric oxide nanozymes. Anal Chem. 2019;91:3827–34.
48. Di H, Mi Z, Sun Y, Liu X, Liu X, Li A, et al. Nanozyme-assisted sensitive profiling of exosomal proteins for rapid cancer diagnosis. Theranostics. 2020;10:9303–14.
49. Oliveira-Rodriguez M. Development of a rapid lateral flow immunoassay test for detection of exosomes previously enriched from cell culture medium and body fluids. J Extracell Vesicles. 2016;5:31803–12.
50. Oliveira-Rodríguez M, Serrano-Pertierra E, García AC, Martín SL, Mo MY, Cernuda-Morollón E, et al. Point-of-care detection of extracellular vesicles: sensitivity optimization and multiple-target detection. Biosens Bioelectron. 2017;87:38–45.
51. Yang Y, Li C, Shi H, Chen TS, Wang ZX, Li GX. A pH-responsive bioassay for paper-based diagnosis of exosomes via mussel-inspired surface chemistry. Talanta. 2019;192:325–30.
52. Wang YM, Liu JW, Adkins GB, Shen W, Trinh MP, Duan LY, et al. Enhancement of the intrinsic peroxidase-like activity of graphitic carbon nitride nanosheets by ssDNAs and its application for detection of exosomes. Anal Chem. 2017;89:12327–33.
53. Xia Y, Liu M, Wang L, Yan A, He W, Lan J, et al. A visible and colorimetric aptasensor based on DNA-capped single-walled carbon nanotubes for detection of exosomes. Biosens Bioelectron. 2017;92:8–15.
54. Chen J, Xu YC, Lu Y, Xing WL. Rapid isolation and visible detection of tumor-derived exosomes from plasma. Anal Chem. 2018;90:14207–15.
55. Chen Z, Cheng SB, Cao P, Qiu QF, Chen Y, Xie M, et al. Detection of exosomes by ZnO nanowires coated three-dimensional scaffold chip device. Biosens Bioelectron. 2018;122:211–6.
56. Xia YK, Wang LL, Li J, Chen XQ, Lan JM, Yan A, et al. A ratiometric fluorescent bioprobe based on carbon dots and acridone derivate for signal amplification detection exosomal microRNA. Anal Chem. 2018;90:8969–76.
57. Hu J, Jiang YZ, Wu LL, Wu Z, Bi Y, Wong G, et al. Dual-signal readout nanospheres for rapid point-of-care detection of ebola virus glycoprotein. Anal Chem. 2017;89:13105–11.
58. Dong D, Zhu L, Hu J, Pang DW, Zhang ZL. Simple and rapid extracellular vesicles quantification via membrane biotinylation strategy coupled with fluorescent nanospheres-based lateral flow assay. Talanta. 2019;200:408–14.
59. Chen XS, Lan JM, Liu YX, Li L, Yan L, Xia YK, et al. A paper-supported aptasensor based on upconversion luminescence resonance energy transfer for the accessible determination of exosomes. Biosens Bioelectron. 2018;102:582–8.
60. Wang Y, Luo D, Fang Y, Wu W, Wang Y, Xia Y, et al. An aptasensor based on upconversion nanoparticles as LRET donors for the detection of exosomes. Sensors Actuators B. 2019;298:126900–7.
61. Lyu Y, Cui D, Huang JG, Fan WX, Miao YS. Near-infrared afterglow semiconducting nano-polycomplexes for multiplex differentiation of cancer exosomes. Angew Chem Int Ed. 2019;58:4983–7.
62. Zhai YZ, Li MX, Pan WL, Chen Y, Li MM, Pang JX, et al. In situ detection of plasma exosomal MicroRNA-1246 for breast cancer diagnostics by a Au nanoflare probe. ACS Appl Mater Interfaces. 2018;104:39478–86.
63. Zheng J, Li NX, Li CR, Wang XX, Liu YC, Mao GB, et al. A nonenzymatic DNA nanomachine for biomolecular detection by target recycling of hairpin DNA cascade amplification. Biosens Bioelectron. 2018;107:40–6.
64. Gao ML, He F, Yin BC, Ye BC. A dual signal amplification method for exosome detection based on DNA dendrimer self-assembly. Analyst. 2019;144:1995–2002.
65. Huang L, Wang DB, Singh N, Yang F, Gu N, Zhang XE. A dual-signal amplification platform for sensitive fluorescence biosensing of leukemia-derived exosomes. Nanoscale. 2018;10:20289–95.
66. Gao M, Yin B, Ye B. Construction of a DNA-AuNP-based satellite network for exosome analysis. Analyst. 2019;144:5996–6003.
67. Wang H, Chen H, Huang ZP, Li TD, Deng AM, Kong JL. DNase I enzyme-aided fluorescence signal amplification based on graphene oxide-DNA aptamer interactions for colorectal cancer exosome detection. Talanta. 2018;184:219–26.
68. Zhou J, Meng LC, Ye WR, Wang QL, Geng SZ, Sun C. A sensitive detection assay based on signal amplification technology for Alzheimer's disease's early biomarker in exosome. Anal Chim Acta. 2018;1022:124–30.
69. Jin D, Yang F, Zhang Y, Liu L, Zhou Y, Wang F, et al. ExoAPP: exosome-oriented, aptamer nanoprobe-enabled surface proteins profiling and detection. Anal Chem. 2018;90:14402–11.
70. Zhang P, He M, Zeng Y. Ultrasensitive microfluidic analysis of circulating exosomes using a nanostructured graphene oxide/polydopamine coating. Lab Chip. 2016;16:3033–42.
71. Zhang QX, Wang F, Zhang HX, Zhang YY, Liu ML, Liu Y. Universal Ti_3C_2 MXenes based self-standard ratiometric fluorescence resonance energy transfer platform for highly sensitive detection of exosomes. Anal Chem. 2018;90:12737–44.
72. Chen H, Luo D, Shang B, Cao J, Wei J, Chen Q, et al. Immunoassay-type biosensor based on magnetic nanoparticle capture and the fluorescence signal formed by horseradish peroxidase catalysis for tumor-related exosome determination. Microchim Acta. 2020;187:282–9.
73. Shi L, Ba L, Xiong Y, Peng G. A hybridization chain reaction based assay for fluorometric determination of exosomes using magnetic nanoparticles and both aptamers and antibody as recognition elements. Microchim Acta. 2019;186:796–800.
74. He F, Wang J, Ye BC. Quantification of exosome based on a copper-mediated signal amplification strategy. Anal Chem. 2018;90:8072–9.
75. Wang L, Yang YC, Liu YF, Ning LM, Xiang Y, Li GX. Bridging exosome and liposome through zirconium–phosphate coordination chemistry: a new method for exosome detection. Chem Commun. 2019;55:2708–11.
76. He DG, Ho SL, Chan HN, Wang HZ, Hai L, He XX, et al. Molecular recognition-based DNA nanodevices to enhance the direct visualization and quantification of single- vesicles of tumor exosomes in plasma microsamples. Anal Chem. 2019;91:2478–775.
77. Wang YY, Liu ZP, Wang XC, Dai YB, Li XY, Gao SF, et al. Rapid and quantitative analysis of exosomes by a chemiluminescence immunoassay using superparamagnetic iron oxide particles. J Biomed Nanotechnol. 2019;15:1792–800.

78. Jiang Q, Liu Y, Wang L, Adkins GB, Zhong W. Rapid enrichment and detection of extracellular vesicles enabled by CuS-enclosed microgels. Anal Chem. 2019;91:15951–8.
79. Zhang HX, Wang ZH, Zhang QX, Wang F, Liu Y. Ti_3C_2 MXenes nanosheets catalyzed highly efficient electrogenerated chemiluminescence biosensor for the detection of exosomes. Biosens Bioelectron. 2019;124-125:184–90.
80. Zhang H, Wang Z, Wang F, Zhang Y, Wang H, Liu Y. In situ formation of gold nanoparticles decorated Ti_3C_2 MXenes nanoprobe for highly sensitive electrogenerated chemiluminescence detection of exosomes and their surface proteins. Anal Chem. 2020;92:5546–53.
81. Zhang Y, Wang F, Zhang H, Wang H, Liu Y. Multivalency interface and g-C_3N_4 coated liquid metal nanoprobe signal amplification for sensitive electrogenerated chemiluminescence detection of exosomes and their surface proteins. Anal Chem. 2019;91:12100–7.
82. Feng Q, Ma P, Cao Q, Guo Y, Xu J. An aptamer-binding DNA walking machine for sensitive electrochemiluminescence detection of tumor exosomes. Chem Commun. 2020;56:269–72.
83. Qiao B, Guo Q, Jiang J, Qi Y, Zhang H, He B, et al. An electrochemiluminescent aptasensor for amplified detection of exosomes from breast tumor cells (MCF-7 cells) based on G-quadruplex/hemin DNAzymes. Analyst. 2019;144:3668–75.
84. Fang D, Zhao D, Zhang S, Huang Y, Dai H, Lin Y. Black phosphorus quantum dots functionalized MXenes as the enhanced dual-mode probe for exosomes sensing. Sensors Actuators B Chem. 2020;305:127544–51.
85. Im H, Shao H, Yong IP, Vanessa MP, Cesar MC, Weissleder R, et al. Label-free detection and molecular profiling of exosomes with a nano-plasmonic sensor. Nat Biotechnol. 2014;32:490–5.
86. Duraichelvana R, Srinivasa B, Badilescua S, Ouelletteb R, Ghoshb A, Packirisamya M. Exosomes detection by a label-free localized surface plasmonic resonance method. ECS Trans. 2016;75:11–7.
87. Giuseppe DN, Bugatti A, Zendrini A, Mazzoldi EL, Montanelli A, Caimi L, et al. Merging colloidal nanoplasmonics and surface plasmon resonance spectroscopy for enhanced profiling of multiple myeloma-derived exosomes. Biosens Bioelectron. 2016;77:518–24.
88. Park J, Im H, Hong S, Castro CM, Weissleder R, Lee H. Analyses of intravesicular exosomal proteins using a nano-plasmonic system. ACS Photonics. 2018;52:487–94.
89. Thakur A, Qiu G, Ng SP, Guan J, Lee Y, Wu CL. Direct detection of two different tumor-derived extracellular vesicles by SAM-AuNIs LSPR biosensor. Biosens Bioelectron. 2017;94:400–7.
90. Wang Q, Zou LY, Yang XH, Liu XF, Nie WY, Zheng Y, et al. Direct quantification of cancerous exosomes via surface plasmon resonance with dual gold nanoparticle-assisted signal amplification. Biosens Bioelectron. 2019;135:129–36.
91. Fan Y, Duan X, Zhao M, Wei X, Wu J, Chen W, et al. High-sensitive and multiplex biosensing assay of NSCLC-derived exosomes via different recognition sites based on SPRi array. Biosens Bioelectron. 2020;154:112066–72.
92. Qiu G, Thakur A, Xu C, Ng S, Lee Y, Wu CML. Detection of glioma-derived exosomes with the biotinylated antibody-functionalized titanium nitride plasmonic biosensor. Adv Funct Mater. 2019;29:1806761.
93. Reiner AT, Ferrer NG, Venugopalan P, Lai RC, Lim SK, Dostalek J. Magnetic nanoparticle-enhanced surface plasmon resonance biosensor for extracellular vesicle analysis. Analyst. 2017;142:3913–21.
94. Stremersch S, Marro M, Pinchasik BE, Baatsen P, Hendrix A, Smedt SCD, et al. Identification of individual exosome-like vesicles by surface enhanced Raman spectroscopy. Small. 2016;24:3292–301.
95. Park J, Hwang M, Choi B, Jeong H, Jung J, Kim HK, et al. Exosome classification by pattern analysis of surface-enhanced Raman spectroscopy data for lung cancer diagnosis. Anal Chem. 2017;89:6695–701.
96. Lee C, Carney R, Hazari S, Smith Z. 3D plasmonic nanobowl platform for the study of exosomes in solution. Nanoscale. 2015;7:9290–7.
97. Lee C, Carney R, Lam K, Chan JW. SERS analysis of selectively captured exosomes using an integrin-specific peptide ligand. J Raman Spectrosc. 2017;48:1771–6.
98. Kwizera EA, O'Connor R, Vinduska V, Williams M, Butch ER, Scott ES, et al. Molecular detection and analysis of exosomes using surface-enhanced Raman scattering gold nanorods and a miniaturized device. Theranostics. 2018;8:2722–37.
99. Ertsgaard C, Wittenberg NJ, Klemme DJ, Barik A, Shih WC, Oh SH. Integrated nanogap platform for sub-volt dielectrophoretic trapping and real-time Raman imaging of biological nanoparticles. Nano Lett. 2018;18:5946–53.
100. Lee JU, Kim WH, Lee HS, Park KH, Sim SJ. Quantitative and specific detection of exosomal miRNAs for accurate diagnosis of breast cancer using a surface-enhanced Raman scattering sensor based on plasmonic head-flocked gold nanopillars. Small. 2019;15(2019):e1804968 1–10.
101. Wang ZL, Zong SF, Wang YJ, Li N, Li L, Lu J, et al. Screening and multiple detection of cancerous exosomes using a SERS-based method. Nanoscale. 2018;10:9053–62.
102. Zong S, Wang L, Chen C, Lu J, Zhu D, Zhang Y, et al. Facile detection of tumor-derived exosomes using magnetic nanobeads and SERS nanoprobes. Anal Methods. 2016;8:5001–8.
103. Ma DD, Huang CX, Zheng J, Tang JR, Li JS, Yang JF, et al. Quantitative detection of exosomal microRNA extracted from human blood based on surface-enhanced Raman scattering. Biosens Bioelectron. 2018;101:167–73.
104. Pang YF, Wang CG, Lu LC, Wang CW, Sun ZW, Xiao R. Dual-SERS biosensor for one-step detection of microRNAs in exosome and residual plasma of blood samples for diagnosing pancreatic cancer. Biosens Bioelectron. 2019;130:204–13.
105. Li TD, Zhang R, Chen H, Huang ZP, Ye X, Wang H, et al. An ultrasensitive polydopamine bi-functionalized SERS immunoassay for exosome-based diagnosis and classification of pancreatic cancer. Chem Sci. 2018;9:5372–82.
106. Fraire JC, Stremersch S, Bouckaert D, Monteyne T, Beer TD, Wuytens P, et al. Improved label-free identification of individual exosome-like, vesicles with Au@Ag nanoparticles as SERS substrate. ACS Appl Mater Interfaces. 2019;11:39424–35.
107. Pang Y, Shi J, Yang X, Wang C, Sun Z, Xiao R. Personalized detection of circling exosomal PD-L1 based on Fe_3O_4@TiO_2 isolation and SERS immunoassay. Biosens Bioelectron. 2020;148:111800.
108. Tian YF, Ning CF, He F, Yin BC, Ye BC. Highly sensitive detection of exosomes by SERS using gold nanostar@Raman reporter@nanoshell structures modified with a bivalent cholesterol-labeled DNA anchor. Analyst. 2018;20:1–84915–22.
109. Liu HY, Weng LY, Yang C. A review on nanomaterial-based electrochemical sensors for H_2O_2, H_2S and NO inside cells or released by cells. Microchim Acta. 2017;184:1–17.
110. Li J, Sculley J, Zhou H. Metal-organic frameworks for separations. Chem Rev. 2012;112:869–932.
111. Furukawa H, Cordova KE, O'Keeffe M, Yaghi OM. The chemistry and applications of metal-organic frameworks. Science. 2013;341:1230444–50.
112. Yang J, Trickett CA, Alahmadi SB, Alshammari AS, Yaghi OM. Calcium L-lactate frameworks as naturally degradable carriers for pesticides. J Am Chem Soc. 2017;139:8118–21.
113. Xiang W, Lv Q, Shi H, Gao L. Aptamer-based biosensor for detecting carcinoembryonic antigen. Talanta. 2020;214:120716.

114. Casal E, Pfaller T, Duschl A, Oostinqh GJ, Puntes V. Time evolution of the nanoparticles protein corona. ACS Nano. 2010;47: 3623–32.
115. Wang D, Lin K, Huang C. Carbon dots-involved chemiluminescence: recent advances and developments. Luminescence 2019;34:4–22.
116. Roda A, Mirasoli M, Michelini E, Fusco MD, Zangheri M, Gevenini L, et al. Process in chemical luminescence-based biosensors: a critical review. Biosens Bioelectron. 2016;76:164–79.
117. Tiwari A, Dhoble SJJT. Recent advances and developments on integrating nanotechnology with chemiluminescence assays. Talanta. 2018;180:1–11.
118. Song H, Su Y, Zhang L, Lv Y. Quantum dots-based chemiluminescence probes: an overview. Luminescence 2019;34:530–43.
119. Sina AAI, Vaidyanathan R, Wuethrich A, Arrascosa LGC, Trau M. Label-free detection of exosomes using a surface plasmon resonance biosensor. Anal Bioanal Chem. 2019;411:1311–8.
120. Zhang R, Zhang Y, Dong ZC, Jiang S, Chen LG, Zhang L, et al. Chemical mapping of a single molecule by plasmon-enhanced Raman scattering. Nature. 2013;498:82–6.
121. Gellner M, Schütz M, Salehi M, Pacheisen J, Strobel P, Marx A, et al. SERS microscopy: plasmonic nanoparticle probes and biomedical applications. Proc SPIE. 2010;7757:77570M.
122. Seballos L, Zhang JZ, Sutphen R. Surface-enhanced Raman scattering detection of lysophosphatidic acid. Anal Bioanal Chem. 2015;383:763–7.
123. Huefner A, Kuan WL, Barker RA, Mahajan S. Intracellular SERS nanoprobes for distinction of different neuronal cell types. Nano Lett. 2013;13:2463–70.
124. Premasiri WR, Lee JC, Sauer-Budge A, Theberge R, Costello CE, Ziegler LD. The biochemical origins of the surface-enhanced Raman spectra of bacteria: a metabolomics profiling by SERS. Anal Bioanal Chem. 2016;408:4631–47.
125. Yoon SS, Lee W, Byun JH, Lee JU. Preparation of graphene/gold nano-hybrid using diamine linker as effective surface-enhanced Raman scattering platforms. J Nanosci Nanotechnol. 2015;15: 8996–9001.
126. Etayash H, McGee AR, Kaur K, Thundat T. Nanomechanical sandwich assay for multiple cancer biomarkers in breast cancer cell-derived exosomes. Nanoscale. 2016;8:15137–41.
127. Cheng N, Song Y, Shi Q, Du D, Liu D, Luo Y, et al. Au@Pd Nanopopcorn and aptamer nanoflower assisted lateral flow strip for thermal detection of exosomes. Anal Chem. 2019;91:13986–93.