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Review

Recent achievements in exosomal biomarkers detection by nanomaterials-based optical biosensors - A review

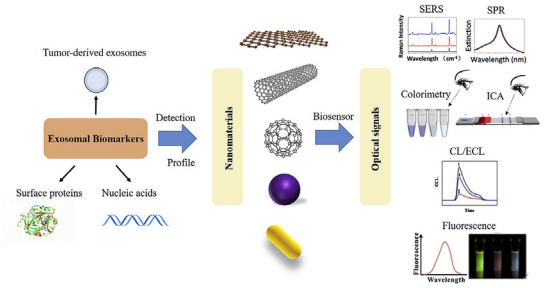
Baoyi Shao, Zhongdang Xiao*

State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, National Demonstration Center for Experimental Biomedical Engineering Education, Southeast University, Nanjing, 210096, PR China

HIGHLIGHTS

- Detection of exosomal biomarkers is a timely topic nowadays.
- Nanomaterial-based optical biosensors for exosomal biomarkers detection are summarized.
- Advantages and disadvantages of different optical biosensors are described.
- Challenges and outlooks for exosomal biomarkers detection are discussed.

GRAPHICAL ABSTRACT



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ABSTRACT

Exosomal biomarkers including tumor-derived exosomes, exosomal surface proteins and exosomal nucleic acids have emerged as one of the most important and general cancer biomarkers in modern biomedical science. These indicators can provide momentous biological information for early diagnosis and treatment of cancer. Recently, numerous studies have been conducted to design biosensors for exosomal biomarkers detection and profiling with high sensitivity and strong applied ability. Among these biosensors, nanomaterial-based optical biosensors are prospective future platforms for rapid and cost-effective detection of exosomal biomarkers. Firstly, we have focused on the progress and advancements in different optical-transducing approaches (Surface-Enhanced Raman Scattering, Surface Plasmon Resonance, Colorimetry, Immunochromatographic assay, Chemiluminescence, Electrochemiluminescence, and fluorescence) for detecting and profiling exosomal biomarkers. Additionally, we have summarized strengths and drawbacks of each strategy. Finally, challenges and future outlooks in developing efficient nanomaterial-based optical biosensor systems for exosomal tumor biomarkers detection have been discussed. The review will exhibit an overview of this field and provide meaningful information for scientific researchers.

Contents

* Corresponding author.
E-mail address: zdxiao@seu.edu.cn (Z. Xiao).

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1. Introduction

1.1. Exosomal cancer biomarkers

Cellular communication is a significant bioprocess of multicellular organisms. In the last few decades, extracellular vesicles (EVs) have been confirmed to exchange biological information and transfer produced molecules between the cells [1,2]. It is increasingly apparent that EVs play a significant role in the regulation of physiological processes, including tissue homeostasis [3], immunity [4], and coagulation [5]. According to pathological processes, EVs have been considered as important factors in diseases such as cancer [6], and neurodegenerative disease [7]. EVs can be divided into three main types: apoptotic bodies (500–2000 nm), microvesicles (50–1000 nm) and exosomes (40–120 nm) [2]. Notably, exosomes have emerged with considerable potential in scientific research due to their small size, which can easily evade the phagocytic system [8,9]. Exosomes are nanosized EVs secreted by cells and present in all bodily fluids (plasma, saliva, urine, ascites, cerebrospinal fluid, etc.) [10], which play an important role in intercellular communication and influence a considerable number of cellular activities in human health and disease [11]. They contain abundant biomolecules from the original cells, including membrane and intracellular proteins, nucleic acids and lipids [12]. These characteristics endow exosomes
with the unique ability to induce different molecular pathways and act as potential and potent biomarkers for early diagnosis of diseases [13]. Exosomal cancer biomarkers refer to cancer markers that are based on cancer-related exosomes. Due to the unique biological properties of exosomes, numerous efforts have been made previously in this area for studying cancer diagnosis and treatment. The exosomal biomarkers can be grouped into three categories: Tumor-derived exosomes (TEXs), exosomal surface proteins, and exosomal nucleic acids [14].

1.1.1. Tumor-derived exosomes (TEXs)

TEXs are released by cancer parent cells; they can alter the cellular physiology of both surrounding and distant normal proliferating cells to promote tumor growth, invasiveness, and metastasis [15,16]. In recent years, many studies demonstrated that the levels of exosomes are markedly high in the bodily fluids of cancer patients, because cancer cells secrete and release markedly more exosomes than non-tumor cells [17–19]. Therefore, TEXs can potentially serve as a biomarker for early diagnosis of tumors as they carry the genetic and typical information of the tumor cells of origin.

1.1.2. Exosomal proteins

Apart from TEXs, numerous specific proteins of exosomes can mirror the derivation and alteration of their mother cells. These proteins contain tetraspanins (e.g., CD63, CD81, CD9), endosome associated proteins (e.g., Rab proteins, annexins, flotillin), heat shock proteins (Hsp 70, Hsp 90, HSPA5, CCT2) and epithelial cell adhesion molecules (EpCAM) [20,21]. Exosomal proteins of TEXs, as potential cancer markers in various cancers including breast, prostate, pancreatic, ovarian, colorectal carcinoma, and glioblastoma [22–27], have emerged for cancer identification and detection over the years. For example, CD24 and EpCAM are typical breast cancer biomarkers that have been discovered recently [24].

1.1.3. Exosomal nucleic acids

In addition to exosomal proteins, exosomal nucleic acids such as microRNAs (miRNAs), mRNAs, tRNAs, lncRNAs and DNAs are also detected in TEXs [28]. Exosomal miRNAs are regarded as the most widespread and conspicuous cancer biomarkers because of their stability against RNase-dependent degradation [29,30]. Exosomal miRNAs were found for the first time in 2007 [31]. In the past decade, several related studies have been reported to use exosomal miRNA as biomarkers in cancer diagnosis. Tanaka et al. have analyzed the expression levels of miRNA-21in blood serum of patients with esophageal squamous cell carcinoma (ESCC) and then discovered the exosomal miR-21 expression is up-regulated in the serum of patients with ESCC compared with the patients without any inflammatory reaction [32]. Taylor et al. have discovered that ovarian cancer cells can be effectively screened from benign cells by the eight specific miRNAs (miR-21, -141, −200a, −200b, −200c, −203, −205, and −214) [33]. These results can well account for the importance of the levels of miRNA in exosomes extracted from the body fluid.

1.2. Determination of exosomal cancer biomarkers

1.2.1. Traditional detection methods

In the last few years, considerable efforts have been made to develop analytical assays for the quantitative detection of exosomal

biomarkers. Approaches for TEXs detection include enzyme-linked immunosorbent assay (ELISA) [34], western blotting [35], flow cytometry [36], and nanoparticle tracking analysis (NTA) [37]. For exosomal proteins, the most common methods are western blotting, ELISA [38], and mass spectrometry [39]. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) [40], microarray [41], and next-generation sequencing [42] have been successfully utilised for the determination of exosomal nucleic acid. Although these techniques are influential and powerful, they still have drawbacks and limitations including sophisticated analysis process, time-consuming operations, and, low sensitivity [43,44]. For example, ELISA require a great deal of samples and complex post-labeling procedure for detection [45]. NTA devices require calibration with beads prior to vesicle concentration and size measurements. The accuracy of TEXs detection is low due to the uncontrollable size of calibrated beads [46]. Under these circumstances, biosensors are suitable and facile analytical tools for the detection of exosomal biomarkers due to their high selectivity, good efficiency, and simple operation.

1.2.2. Optical nanomaterial-based biosensor

Sensitivity and specificity are two essential standards of measurement for a biosensor. Fundamental biosensor comprises a biological recognition section that can capture the targeted analyte and a sensing section that can change the biological variation into physical or chemical signals [47,48]. The sensitivity is primarily influenced by the receptor such as antibody, nucleic acid (*eg.* aptamer), enzyme, microbe, and receptor immobilization process, appropriate substrate and sensing approach, while the specificity is affected by an available combination between biological recognition and sensing sections [49]. In recent years, nanomaterials with unique optical properties including Au, Ag nanoparticles [50], graphene [51], carbon nanotubes [52], and quantum dots [53] have been widely used to construct optical substrates to increase the sensitivity of target detection. For instance, metallic nanoparticle is used to apply in SERS-based detection for signal enhancement. Quantum dots are considered as an ideal fluorophore for fluorescent detection with a wide absorption band and high resistance to photobleaching. In general, many types of nanomaterials play diverse roles in the biosensor-based system. The development of optical biosensors based on nanomaterials for targets sensing is an on-going and novel trend in the area of analytical diagnostics [54]. To detect exosomal cancer biomarkers, nanomaterials-based optical biosensor has become a promising and useful analysis tool for cancer diagnosis due to their high sensitivity, simplicity of procedure, and rapid time of detection. Exosomal cancer biomarkers optical techniques are categorized based on the different optical detection methods used, mainly consisting of Surface enhanced Raman Scattering (SERS), Surface Plasmon Resonance (SPR), colorimetric, Immunochromatographic assay (ICA), Chemiluminescence (CL), Electrochemiluminescence (ECL), and fluorescence.

In this review, we focus on current progress in exosomal cancer biomarkers detection using diverse optical biosensors based on nanomaterials (Fig. 1). Next, the advantages and disadvantages of each optical biosensor are summarized. In addition, future direction and perspective of exosomal cancer biomarkers detection are also discussed in the last part of the article. Our aim is to provide the readers with a comprehensive notion of optical nanomaterial-based biosensor for the detection of exosomal cancer biomarkers and, more importantly, an expectation of an increasing number of cost-effective test methods that can be used for the early diagnosis of cancer in the future.

2. Current optical nanomaterial-based biosensors

2.1. Surface-enhanced Raman scattering

Plasmon resonances are aroused by the interaction of light and noble metal surfaces in nanoscale, that have been widely used in the area of biosensor. Surface-enhanced Raman scattering (SERS), as a powerful plasmonic technique, has been widely applied in biomedical analytical field. It has been used for the detection of biomolecules, cellular imaging, and environmental monitoring [55,56]. Enhancement of Raman intensity relies on localized surface plasmon resonance, which can generate a strong electromagnetic field among metallic nanoparticles. Particular fingerprint information can be provided by Raman spectra with high sensitivity and favorable stability [57,58].

SERS nanomaterial-based biosensors have been successfully applied to detect exosomal biomarkers due to their rapid speed, high sensitivity and multiplexing ability. Wang and co-workers have designed a SERS-based biosensor for simultaneous multiple detections of TEXs using SERS probes (Au nanoparticles decorated with a Raman reporter and aptamer for particular exosome) and capturing substrates (magnetic beads with Au shell modified by CD63 aptamer) [59] (Fig. 2a). In their work, they produced three types of SERS probes to bind specifically to SKBR3, T84, and LNCaP exosomes at the same time and then used capturing substrates to achieve signal separation. Decrease of Raman intensities in the supernatant was analyzed to obtain a standard curve for detecting TEXs. The LOD values of this method are 32, 73, and 203 particles per microliter for the SKBR3, T84, and LNCaP exosomes, respectively. Additionally, the assay was established to detect the real blood samples acquired from cancer patients.

Similarly, exosomal surface proteins can be identified by SERS. Kwizera et al. have produced a novel SERS-based biosensor for exosomes detection and protein profiling [60]. Firstly, a 3D-printed array template attached onto a glass slide coated with Au film was designed to capture exosomes by using antibodies. Secondly, Au nanorods modified with Raman reporters as SERS tags were bound to the surface of exosomes in order to enhance Raman intensity for effective detection. In this experiment, this SERS-based biosensor was applied in three aspects. (1) Exosomes were able to be detected sensitively and specifically. (2) Exosomes derived from different types of breast cancer cells and normal breast cells were compared to identify the characteristic surface proteins which could mirror the protein profile of parent cells. (3) HER2 and EpCAM biomarkers on exosomes were measured in blood serum from HER2-positive breast cancer patients. This method was easy, low-cost and portable for cancer diagnosis and monitoring.

Furthermore, SERS can be applied for exosomal nucleic acid detection. Sim and co-workers have fabricated a SERS biosensor based on head-flocked Au nanopillars substrate for the detection of exosomal microRNA [61]. Firstly, locked nucleic acid (LNA), a short complementary oligonucleotide of microRNA, captured probes that could hybridize to the target microRNA were immobilized on the plasmonic gold nanopillar substrate. When the SERS substrate was immersed in solvents, adjacent nanopillars would be closer, driven by capillary effects, which could enhance SERS signals. Next, Cy3-LNA detection probes were used to distinguish the target microRNA for ultrasensitive detection. In the assay, this SERS-based sensing system was able to observe the expression levels of microRNA in breast cancer cell lines and screen breast cancer types according to the results of different exosomal microRNA detection. In addition, Pang and co-workers have reported a one-step method for the determination of microRNAs in exosome [62] (Fig. 2b). In their experiment, Fe_3O_4@Ag magnetic nanoparticles were used as capture probes, Au core @Ag shell nanostructures with Raman reporter were used as SERS tags.

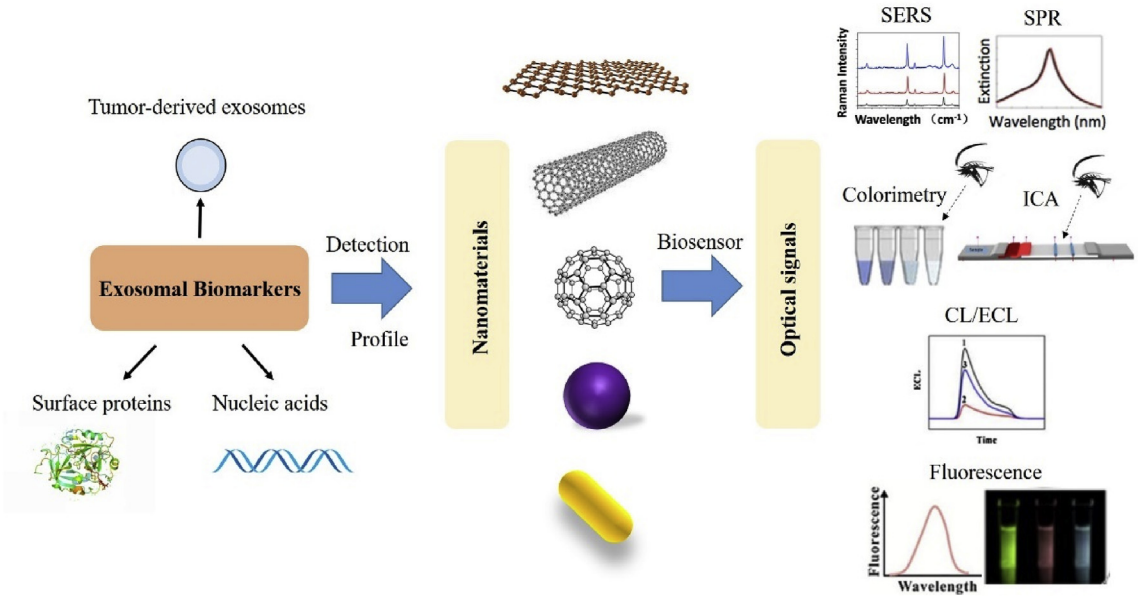

Fig. 1. Overview of the process and principle of nanomaterial-based optical biosensors for the detection of exosomal biomarkers. Abbreviations: SERS, surface-enhanced Raman scattering; SPR, surface plasmon resonance; ICA, Immunochromatographic assay; CL, Chemiluminescence; ECL, Electrochemiluminescence.

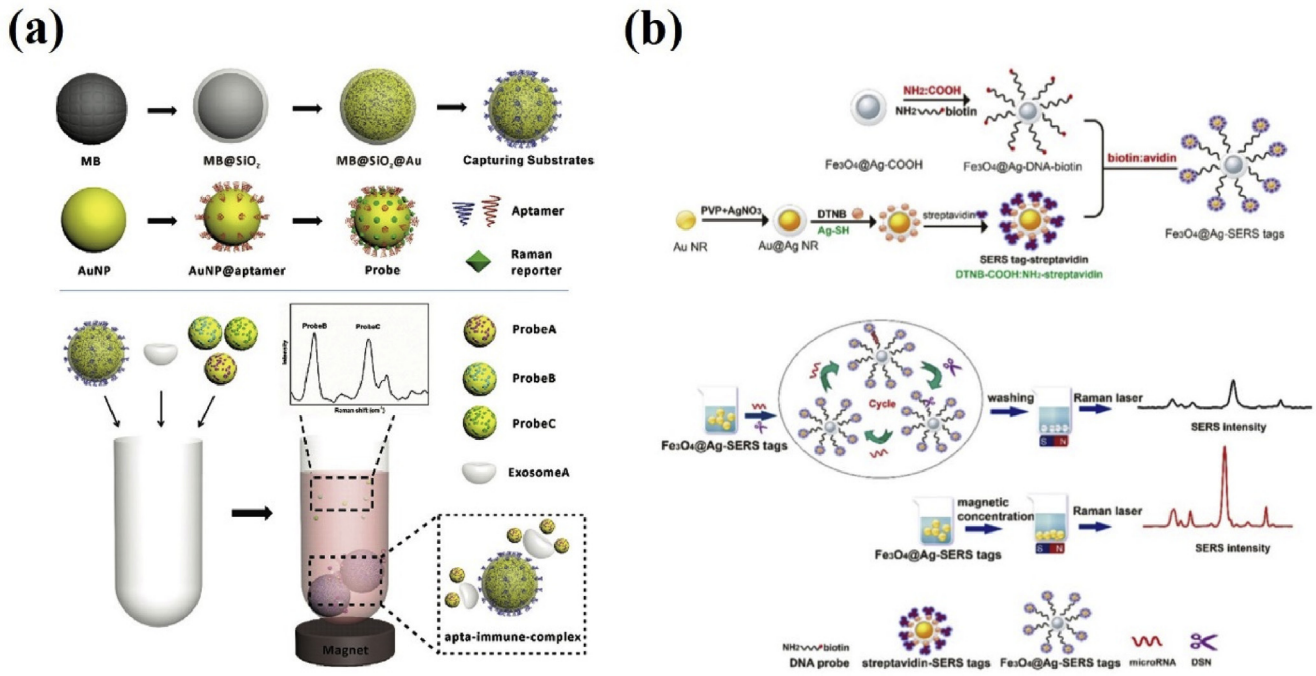

Fig. 2. Representative SERS biosensors. (a) Detection of TEXs based on Au nanoparticles and magnetic beads with Au shell. (Reproduced with permission from Ref. [59] Copyright 2018 Royal Society of Chemistry). (b) Detection of microRNA-10b based on Fe_3O_4@Ag-DNA and Au@Ag@DTNB. (Reproduced with permission from Ref. [62] Copyright 2019 Elsevier). Abbreviations: MB, magnetic nanobeads; AuNP, Au nanoparticle.

Exosomal microRNA-10b was detected precisely in blood samples based on dual-SERS biosensor (LOD = 1.0 aM). As a result, exosomal microRNAs could be detected with effects by using SERS-based biosensor, which was meaningful for early cancer diagnosis.

2.2. Surface plasmon resonance (SPR)

Surface plasmon resonance (SPR) is a plasmonic analytical method that has appeared in the past few years as an efficient and dependable platform in clinical detection for interactions of biological molecules [63,64]. SPR in the visible and near-infrared wavelength range are of specific interest because there is effective optical confinement at these wavelengths [65,66]. Basically, the resonance condition for an assigned plasmonic configuration is exceedingly sensitive to the changes of refractive index in nanoscale from the metal material [66]. Based on the distinctive and simple optical phenomenon, diverse biological receptors including aptamer, antibody, and enzyme are usually immobilized on the

surface of a metal to form SPR biosensors [67]. Targets can be detected with no difficulty based on the SPR response to the conjugation reaction [68]. SPR nanomaterial-based biosensors have attracted extensive attention in biomarker detection due to their ability to realize the label-free and real-time measurement [69].

Wang and coworkers have reported a SPR nano-based aptsensor for exosomes detection with high sensitivity and excellent specificity [70] (Fig. 3a). In the first place, the target exosomes were captured by DNA which was modified in the gold film. Next, aptamer (T_{30}) coated AuNPs were connected with target exosomes, up to now, a single AuNP amplified SPR biosensor was designed. A_{30}, a complementary sequence of T_{30}, was functionalized on the surface of AuNPs, which could capture the T_{30} coated AuNPs by the principle of complementary base pairing. In the end, a dual AuNP amplified SPR biosensor was structured and target exosomes were detected efficiently and usefully. This promising SPR-based biosensor has provided a new idea for the quantification of exosomes and offered an avenue for cancer diagnostics and clinical detection.

Wu and co-workers have reported a SPR biosensor with self-assembly gold nanoislands (SAM-AuNIs) for the detection and distinguish of two different cancer exosomes (A-549, SH-SY5Y) [71] (Fig. 3b). As an SPR-active sensing substrate, the structure of SAM-AuNIs was constructed by a two-step deposition-annealing approach for the detection of exosomes and distinguishing exosomes from microvesicles without any functionalization. The biosensor obtains the LOD value to 0.194 μg/mL. Later, their group has devised a SPR biosensor using Titanium nitride (TiN), a great plasmonic ceramic material, for glioma-derived exosomal proteins detection [72]. Compared with gold and silver, TiN demonstrated adjustable and steady electroconductibility and has plasmonic property in near infrared visible spectrum. In the assay, TiN film was functionalized by biotinylated anti-CD63 or anti-epidermal growth factor receptor variant-III (anti-EGFRvIII) antibodies due to the high affinity of biotin-TiN interaction. CD63 marker and EGFRvIII of exosomes could be captured by anti-CD63 antibodies and anti-EGFRvIII respectively, which composed a SPR-based TiN biosensing system for detection sensitively and selectively. The LOD values of CD63 and EGFRvIII are 4.29×10^{-3} and 2.75×10^{-3} μg mL^{-1}respectively. The results exhibited that the biosensor based on TiN had better sensitivity than conventional gold-film biosensor. Furthermore, this proposed SPR biosensor could be used as an effective tool to quantify exosomal cancer biomarkers in the body fluid of patients.

In terms of exosomal protein profiling, Im and co-workers have described a nano-plasmonic exosome (nPLEX) method based on SPR by gold layer with periodic nanohole arrays [73]. Each array is modified with specific antibodies for diverse exosomal surface proteins profiling (71 protein markers in ovarian cancer cell lines and benign cells). This biosensor could be promising tool to explore exosomal cancer biomarkers for early diagnostics and therapeutic evaluation.

2.3. Colorimetry

Colorimetry has been ancient and simple analysis method all along, because the results can be easily observed with our naked eyes [74]. Colorimetric biosensor has been demonstrated to be a promising technique to detect exosomal biomarkers by based on the high extinction coefficient to realize quick analysis. Nanomaterial-based colorimetric biosensor can be categorized into two groups based on different properties of materials: innate optical properties (such as colloidal AuNPs) and catalytic properties (such as Fe_3O_4 NPs).

AuNPs are widely applied in colorimetric assays that rely on the change of color generated by pellet aggregation [75]. For instance, Liu et al. have constructed an AuNP-based colorimetric biosensor for TEXs detection [76] (Fig. 4a). Depending on double proximity ligation assay (PLA) probes that can capture TEXs, they designed a distinctive fungible DNA signal for the specific biomarker, which was synchronously amplified twice by recombinase polymerase amplification (RPA) as well as transcription-mediated amplification (TMA). This so-called PLA-RPA-TMA assay was able to detect quantitatively $LMP1^+$ (LOD = 10^2 particles mL^{-1}) and $EGFR^+$ exosomes (LOD = 10^2 particles mL^{-1}). The AuNP-based colorimetric biosensor can also be used in profiling exosomal proteins for cancer diagnosis. Jiang et al. have designed an AuNP-based biosensor platform that assesses exosome surface proteins rapidly by the naked eye [77]. In this assay, AuNPs were modified with the aptamers of exosomal protein (CD63). The

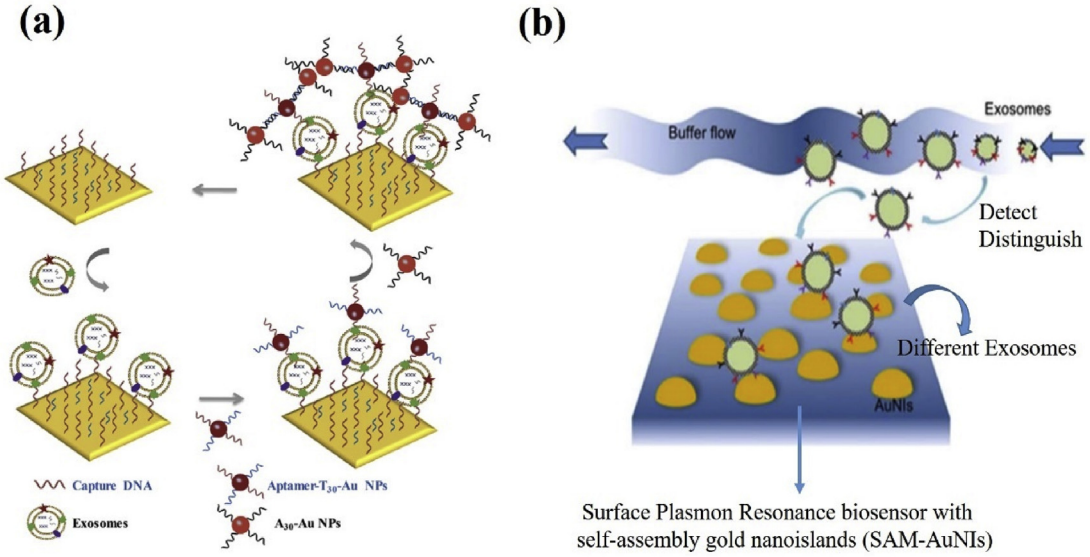

Fig. 3. Representative SPR biosensors. (a) Detection of TEXs based on dual AuNP-assisted signal amplification. (Reproduced with permission from Ref. [70] Copyright 2019 Elsevier). (b) Detection of TEXs based on Au nanoparticles and magnetic beads with Au shell. (Reproduced with permission from Ref. [71] Copyright 2017 Elsevier). Abbreviations: AuNPs, Au nanoparticles; AuNIs, Au nanoislands.

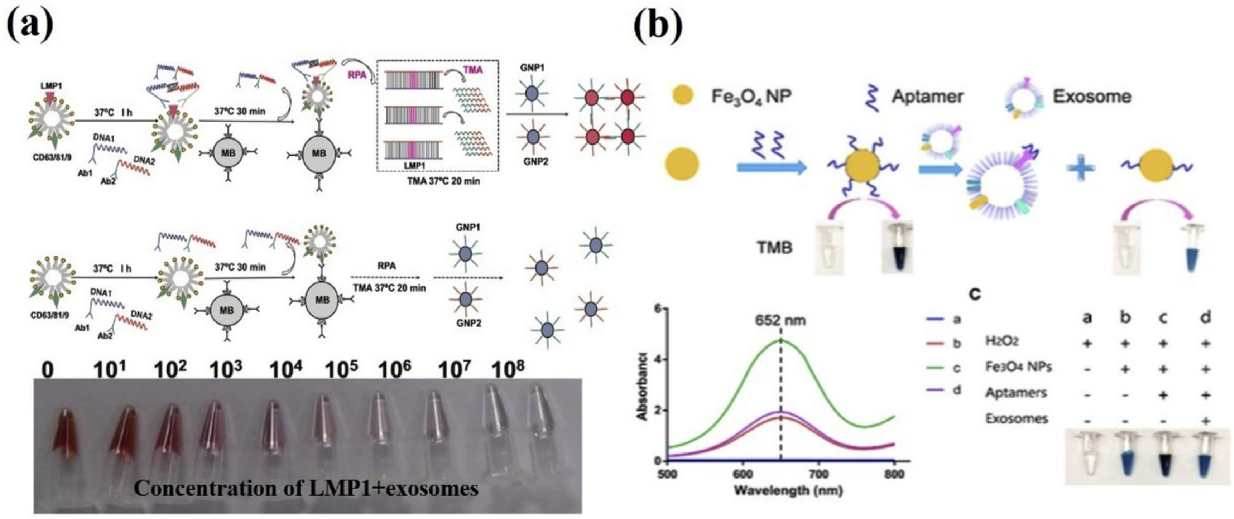

Fig. 4. Representative colorimetric biosensors. (a) Detection of TEXs based on AuNPs with PLA-RPA-TMA method. (Reproduced with permission from Ref. [76] Copyright 2018 Elsevier). (b) Detection of TEXs based on aptamer-capped Fe_3O_4 nanoparticles. (Reproduced with permission from Ref. [78] Copyright 2018 American Chemical Society). Abbreviations: MB, magnetic beads; GNP, gold nanoparticle; RPA, recombinase polymerase amplification; TMA, transcription-mediated amplification; Fe_3O_4NP, Fe_3O_4 nanoparticle; TMB, 3,3′,5,5′-tetramethylbenzidine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

complexation of aptamers could avoid the nanoparticles to aggregate in a high concentration of NaCl. In the existence of exosomes, AuNP would disconnect with aptamers, and it would result in the aggregation of the AuNPs (color change). This method was able to profile different exosome surface proteins (CD63, EpCAM, PDGF, PSMA, and PTK7) from diverse cell lines (HeLa, PC-3, CEM, and) Ramos) within minutes.

Another type of colorimetric biosensor is based on the catalytic property. For example, Chen and coworkers have reported a visual, label-free and easy method with aptamer-functionalized magnetite nanoparticles (Fe_3O_4 NPs) to detect PC3-derived exosomes in blood [78] (Fig. 4b). The principle of this colorimetric biosensor is that the aptamer of epithelial-cell-adhesion molecule (EpCAM) can apparently enhance the peroxidase activity of Fe_3O_4 NPs, catalyzing TMB oxidation for a change of color (from colorless to varying degree of blue). The value of LOD is 3.58×10^6 particles mL^{-1}.

2.4. Immunochromatographic assay (ICA)

Immunochromatographic assay (ICA) is a paper-based test strip method with the advantages of low cost, simple procedure, and fast detection speed. ICA test strip is composed of a sample pad, a conjugate pad, a nitrocellulose filter, and an absorbent pad [79]. As labels of ICA, numerous nanomaterials including colloidal AuNPs, UCNPs, and, QDs have been used in test strips to observe the interactions between targets and receptors. Colloidal AuNPs are the most popular nanomaterials in ICA. Wu and coworkers produced a dual AuNPs biosensor of exosomes based on lateral flow assay [80]. CD9 antibodies which could capture exosomes were modified on 15 nm-sized AuNPs. 40 nm-sized AuNPs were used to bind to the 15 nm-sized AuNPs and achieve signal amplification. The colorimetric biosensor was able to detect MCF-7 cell-derived exosomes rapidly with LOD of 1.3×10^3 particles μL^{-1} and also found to be suitable in fetal bovine serum.

2.5. Chemiluminescence (CL)/Electrochemiluminescence (ECL)

Chemiluminescence (CL) is the emission of light based on chemical reaction. The chemiluminescent reaction does not need an excitation light source for target analytes radiation, thus this approach is extensively used in the field of optical biosensor [81]. Recently, only a few CL-based approaches combined with nanomaterials for the detection of exosomal biomarkers have been reported. Wang et al. have established a novel CL-based biosensor for the rapid quantitative detection of TEXs from biofluids based superparamagnetic iron oxide particles (SIOPs) [82]. Anti-CD63 antibodies and acridinium ester (ACE)-labeled anti-CD9 antibodies with great chemiluminescent property were decorated on the surface of SIOP. As a capture probe, antibody modified SIOP could recognize the TEXs forming a biosensing system for a one-step test. The LOD value of this CL-based biosensor was 2.63×10^5 particles μL^{-1}.

Electrochemiluminescence (ECL) is another widely-used detection assay that combines the superiorities of electrochemical biosensors with the shining points of CL-based biosensors. ECL-based biosensor basically relies on an electron transfer reaction at the surface of electrodes which can test samples more effectively [83,84]. Several nanomaterials with excellent ECL performance have been explored in analytical applications such as QDs, CNTs, silica nanoparticles (SiNPs), metallic nanoparticles, and g-C_3N_4 nanosheets [84]. Recent works on the detection of exosomal biomarkers are not many either. For instance, Zhang et al. have developed an ECL-based biosensor with aptamer-functionalized Ti_3C_2 MXenes nanosheets (a two-dimensional nanomaterial) for TEXs quantitative detection [85]. In the experiment, the cancerous exosomes derived from MCF-7 can be captured onto the electrode surface which decorated with EpCAM-aptamers. This biosensing system can detect MCF-7 exosomes with high efficiency and sensitivity. The LOD is 125 particles μL^{-1}, which was far below the traditional ELISA approach. Furthermore, the biosensor could be successfully applied to the detection of TEXs in serum.

2.6. Fluorescence

Fluorescence-based biosensor is a significant and popular detection technique which is extensively applied in analytes detection with high sensitivity and strong operability. In recent years, nanomaterials, including two-dimensional nanomaterials, QDs, CNTs, UNCPs, metallic nanoparticles, MNPs, and others have been applied in biosensor based on fluorescence resonance energy

transfer (FRET) due to their distinctive optical properties [86,87].

Among these materials, two-dimensional nanomaterials such as Ti_3C_2, graphene oxide (GO) and MoS_2 have emerged as a major part in FRET-based biosensing platform. Zhang and colleagues have presented a characteristic fluorescence-based biosensor based on the CD63 aptamer labeled with Cy3 dye (CD63-Cy3 aptamer) and Ti_3C_2 nanosheet for quantitative detection of exosomes [88] (Fig. 5a). CD63-Cy3 aptamer could be modified on the surface of the Ti_3C_2 nanosheet by hydrogen bonding. The fluorescence signal of the Cy3 dye would be quenched by the chelation effect between the phosphate group of aptamers and Ti ions on the nanosheet. With the existence of target exosomes, the Cy3-CD63 aptamers would release from the Ti_3C_2 nanosheet due to the high affinity between Cy3-CD63 aptamer and CD63 peripheral protein. Therefore, the fluorescence of Cy3 dye was recovered promptly. The LOD of HepG2 exosomes detection on the basis of this biosensor is 1.4×10^3 particles mL^{-1} and thus it is highly sensitive and greatly practical. In addition, the sensor was used to reorganize three different markers and four types of cancerous exosomes. GO is another suitable and frequently-used 2D-nanomaterial which is used in the exosomal biomarkers application. For example, Jin and coworkers developed a novel fluorescent biosensor for profiling of exosomal surface proteins and quantitative detection of exosomes based on GO and different aptamers (Fig. 5c) [89]. In their experiment, this suggested biosensor could detect exosomes (LOD = 1.6×10^5 particles mL^{-1}) and also profile surface proteins of exosomes from five cell types. The results in this assay also demonstrated the preponderance of 2D-nanomaterials, which are applied in fluorescence-based biosensor.

Apart from 2D-nanomaterials, metallic nanoparticles are also an ideal option. Duan's group has fabricated an AuNP probe-based biosensor for exosomal microRNA-1246 in plasma [90] (Fig. 5b). The AuNP probe, which was composed of AuNP, Cy5-labeled DNA and capture DNA (complementary sequence of Cy5-labeled DNA), could enter into exosomes with the fluorescence signals quenched initially. Capture DNA was then bound to exosomal microRNA-1246 and fluorescence signals were recovered simultaneously due to the free Cy5-labeled DNAs.

Liposomes, closely resemble exosomes and have great biocompatibility, appropriate size and facile surface functionalization. Liposome-based fluorescent signal amplification systems for exosomal biomarkers detection have attracted wide attention these days. Wang and colleagues have produced a liposomes-based sandwich structure for quantitative detection of exosomes [91]. The fluorescent biosensor platform is composed of CD63 antibody, magnetic beads (MBs), exosomes, zirconium ions (Zr^{4+}) and liposomes loaded with calcein. At first, CD63 antibody decorated MBs were used for the acquisition of exosomes and Zr^{4+} could be absorbed by exosomes based on the interaction of coordination chemistry between Zr^{4+} and phosphate. Afterwards, liposomes with calcein were added into the aforementioned system to mold the sandwich structure. Lastly, the fluorescence signal in the suspension based on magnetic separation had a positive correlation to the concentration of exosomes. As a result, the biosensor could detect TEXs easily and sensitively.

3. Comparison of different nanomaterial-based optical biosensors

There are seven current optical approaches based on nanomaterials (SERS, SPR, colorimetry, ICA, CL, ECL, and fluorescence)

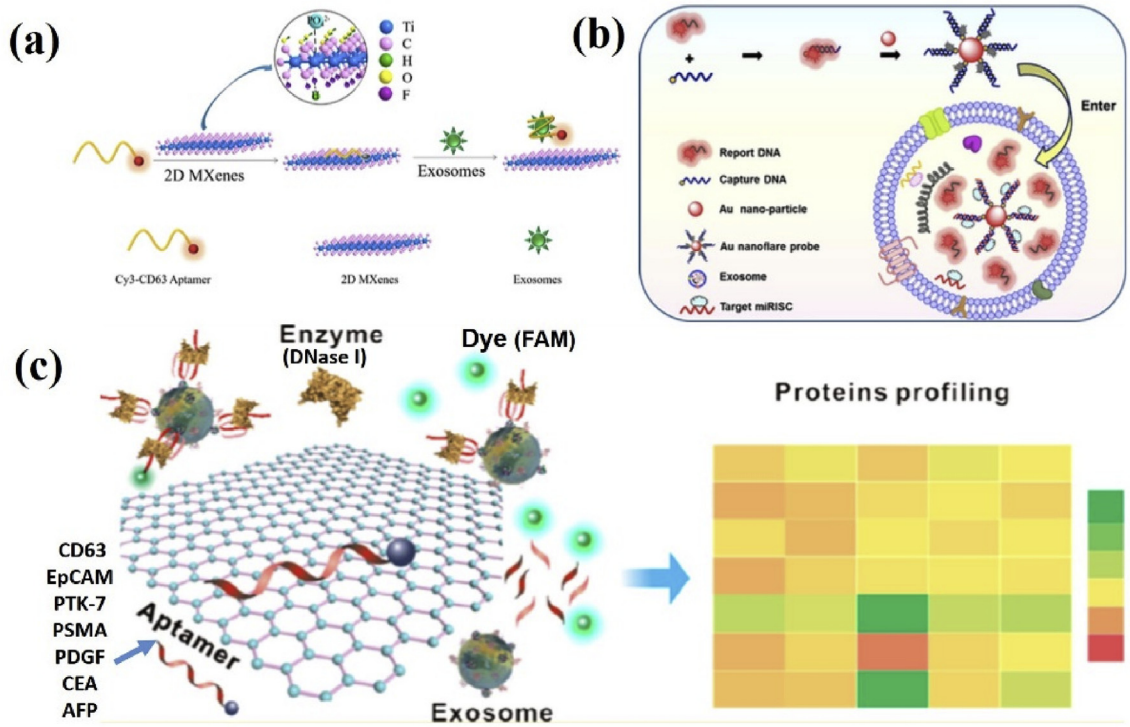

Fig. 5. Representative fluorescent biosensors. (a) Detection of TEXs based on Ti3C2 MXene nanosheets with Cy3-CD63 aptamer. (Reproduced with permission from Ref. [88] Copyright 2018 American Chemical Society). (b) Detection of microRNA-1246 based on spherical nucleic acid functionalized Au nanoflare. (Reproduced with permission from Ref. [90] Copyright 2018 American Chemical Society). (c) Profiling of surface markers and detection of exosomes based on an aptamer-confined nanoprobe that complements with enzyme-mediated signal amplification for. (Reproduced with permission from Ref. [89] Copyright 2018 American Chemical Society). Abbreviation: 2D MXenes, two-dimensional transition-metal carbides and carbonitrides materials; FAM, 5-carboxy fluorescein; EpCAM, epithelial cell adhesion molecule; PTK-7, protein tyrosine kinase 7; PSMA, prostate-specific membrane antigen; PDGF, platelet-derived growth factor; CEA, carcinoembryonic antigen; AFP, α-fetoprotein; DNase I, deoxyribonuclease I.

Table 1
Merits and shortcomings of different kinds of biosensor.

Types of Biosensor	Merits	Shortcomings
SERS	High sensitivity, nondestructive analysis, real-time monitoring	Poor repeatability, costly equipment
SPR	Real-time monitoring, label-free, high sensitivity	suffers from nonspecific adsorption
Colorimetry	Simplicity, easy operation, low cost	low sensitivity
ICA	Simplicity, easy operation, low-cost, portability	low sensitivity
CL/ECL	Simplicity, low cost, high sensitivity	Interferences of background absorption
Fluorescent	Extremely high sensitivity	Expensive instruments, interferences of background fluorescence and quenching effect

Table 2
Summary of representative nanomaterial-based biosensors for exosomal biomarkers detection.

Method	Nanomaterials	Exosomal marker	Cell lines	LOD	References
SERS	ARANPs-SiMB	miRNA-21	HeLa, A549,	5.0 fM	[92]
	Fe_3O_4@Ag NPs -Au@Ag NP	MiRNA-10b	293	1.0 aM	[62]
	Gold nanopillar	miRNA-21	PANC-01, HPDE6-C7, MCF-7,	1.0 aM	[61]
	AuNRs	miRNA-222	MCF-10	2×10^6 particles μL^{-1}	[60]
	AuNS@Au-MBs	miRNA-200c	Breast cell lines	27 particles μL^{-1}	[93]
		TEXs	MM468,		
		TEXs	SKBR3,		
			MCF12A		
			HepG2		
SPR	MB@SiO2@Au	TEXs	SKBR3, T84,	32 particles μL^{-1}	[59]
	-AuNPs	TEXs	LNCaP	73 particles μL^{-1}	[70]
	AuNPs-Au film	TEXs	MCF-7,	203 particles μL^{-1}	[71]
	AuNIs	CD63,	A549,	5×10^3 particles mL^{-1}	[72]
	TiN	EGFRvIII	SH-SY5Y	0.194 $\mu g\ mL^{-1}$	
			U251	4.29×10^{-3} $\mu g\ mL^{-1}$	
				2.75×10^{-3} $\mu g\ mL^{-1}$	
Colorimetric	Gold layer	TEXs	SH-SY5Y	Not mentioned	[94]
	Gold surface	TEXs	MHCC97L	Not mentioned	[95]
	Gold film with periodic nanoholes	71 exosomal protein markers	MHCC97H	Not mentioned	[73]
	Fe_3O_4 NPs	TEXs	B16–F1/B16–F10	3.58×10^6 particles mL^{-1}	[78]
	g-C_3N_4 NSs	TEXs	Ovarian cancer cell/Benign lines	13.52×10^5 particles mL^{-1}	[96]
	ZnO NWs	TEXs	PC3	2.2×10^4 particles μL^{-1}	[97]
	AuNPs	TEXs	HeLa	10^2 particles mL^{-1}	[76]
	SWCNTs	TEXs	MCF-7	5.2×10^5 particles μL^{-1}	[98]
		TEXs	PC3		
			MCF-7		
			MCF-7		
			NPC		
			MCF-7		
ICA	AuNPs	TEXs	MCF-7	1.3×10^3 particles μL^{-1}	[80]
	QDs	TEXs	Cal27	2.0×10^3 particles μL^{-1}	[99]
Fluorescent	CDs	miRNA-21	MCF-7,	3.0 fM	[100]
	AuNPs	miRNA-1246	MDA-MB-231	0.68 nM	[90]
	CuONPs, CuNPs	TEXs	MCF-7	4.8×10^4 particles μL^{-1}	[101]
	Fe_3O_4 NP	TEXs	HepG2	100 particles μL^{-1}	[103]
	AuNP	TEXs	PSA	1.16×10^3 particles μL^{-1}	[102]
	GO	TEXs	PCa	2.1×10^4 particles μL^{-1}	[104]
	GO	TEXs	HepG2	1.6×10^5 particles μL^{-1}	[89]
	Liposomes, Zr^{4+}	TEXs	SW480	7.6×10^3 particles μL^{-1}	[91]
	Ti3C2 MXenes	TEXs	HepG2	1.4×10^3 particles mL^{-1}	[88]
	UCNP-AuNRs	TEXs	HeLa	1.1×10^3 particles μL^{-1}	[105]
			SGC7901		
			HeLa		
			B16, MCF-7,		
			OVCAR-3, HepG2		
			HepG2		
CL	SIOP	TEXs	HepG2	2.63×10^5 particles μL^{-1}	[82]
ECL	Ti3C2 MXenes	TEXs	MCF-7	125 particles uL^{-1}	[85]

Abbreviations: ARANPs, Au@R6G@AgAu nanoparticles; SiMBs, Silica microbead; MBs, Magnetic nanobeads; AuNRs, Au nanorods; AuNSs, gold nanostars; AuNPs, gold nanoparticles; AuNIs, gold nanoislands; TiN, Titanium nitride; ZnO NWs, ZnO nonwires; SWCNTs, Single-walled carbon nonotubes; g-C_3N_4 NSs, Graphotic carbon nitride nanosheets; CDs, Carbon dots; SIOPs, Superparamagnetic iron oxide particles; UCNP, Upconversion nanoparticles.

used to detect and profile exosomal biomarkers. Each optical biosensor has its own distinctive characters. The advantages and disadvantages are listed in Table 1. In general, increasing the sensitivity and selectivity of different biosensors are the most fundamental and significant objectives in the entire biosensing system.

In the past decades, colorimetric and fluorescence techniques have been the most widespread signal transduction modes among these optical biosensors. Based on the LOD values of different optical techniques (Table 2), fluorescent biosensors showed relatively high sensitivity compared to other methods for the detection of cancer biomarkers. The high sensitivity of fluorescent biosensing

platform is due to low background noise and unique optical properties of novel nanomaterials (GO, UCNP, AuNPs). Colorimetry, another popular test method, generally exhibit a lower sensitivity than fluorescent assays, however it can show the results of detection in a more intuitive and simpler way. To some degree, ICA is a kind of colorimetric method based on a solid platform. ICA strips with nanomaterials labels are convenient to carry and can be used commercially. SERS and SPR biosensors both depend on plasmonic effect; therefore, they have a lot in common such as real-time detection, high sensitivity, etc. For the detection of biological targets, comparatively speaking, SERS biosensor is more advantageous due to the unique fingerprint vibration spectrum without any damage. With regard to CL and ECL, only limited research and application are carried out in exosomal biomarker detection. However, these techniques have broadened the way of thinking for a scientific researcher in this area.

4. Challenges

We have reviewed the most recent advances in nanomaterial-based biosensors for detecting different exosomal cancer biomarkers with regard to different optical signal transduction techniques. Although the development of nanomaterial-based optical biosensors for exosomal cancer biomarker detection has markedly progressed in recent years, there are still some challenges to be surmounted about the sensing system.

(1) Stability of biosensors and exosomes: Despite the sensitivity of designed biosensors that can even reach to Pico molar, low stability is a major problem for continuous detection of the target efficiently. Owing to the instability of chemical modification with chemical groups, including thiol, amino, antibody, aptamer and fluorescence dyes, many nanomaterial-based biosensors are unsuitable for long time storage. Therefore, efforts need to be aimed at exploring the optimum storage conditions in the light of diffident chemical modification with groups. In addition, signals of biosensors will change due to poor stability of synthesized nanomaterials. Further studies are required to examine the design of biosensors, such as how to improve stability of nanoparticles, how to make the modified groups more stable, and how to create controllable biosensors to improve stability. Moreover, the stability of exosomes is dependent on storage conditions mainly including temperatures, and time. In most cases, below −70 °C is the optimum temperature for the storage of exosomes for clinical application and fresh exosomes are the best option. However, the conditions that affect the preservation of exosomes are diverse and complex. It is a worthy research direction to explore the optimum conditions required for exosomes analysis.

(2) Specificity of biosensors and exosomal biomarkers: The selective specificity of biosensors for exosomal cancer biomarker detection is generally based on functionalized receptors such as antibody and aptamer. In view of this, the screening of antibody or aptamer is particularly important in the whole experiment. Nevertheless, the cost of screening is high and the specificity is barely satisfactory. It is essential to develop a cost-effective technique for antibody and aptamer screening. Besides, the selection of specific exosomal biomarkers according to the different requirements for early cancer diagnosis is a great challenge in specific detection. If there is not an ideal single exosomal biomarker with a clear diagnostic attribute, multiplex exosomal biomarkers would be required to be analyzed simultaneously with low efficacy in one biosensor system.

(3) Clinical application: The results of detection will have clinical significance only by acquiring data from bodily liquids. However, it is an enormous challenge to test exosomal biomarkers precisely in most bodily liquids due to their complex ingredients. Improving the efficiency and accuracy of the designed biosensor in bodily liquids for applying in cancer diagnosis are also required.

5. Future outlook

Exosomal biomarkers are being widely considered as idea therapeutic targets for oncotherapy. For exosomal biomarkers detection, future efforts should focus on synthetizing multifunctional nanomaterials and making the optical biosensors more sensitive and timesaving. More importantly, for further application in blood serum which represents complex conditions, the detection system should also be designed meticulously, i.e. improving biorecognition activity. Certainly, we believe the research in the detection of exosomal cancer biomarkers using nanomaterial-based optical biosensors will play increasingly significant roles in the area of human health and eventually become practical and effective analytic tool that could meet many challenges in the future.

Declaration of competing interest

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

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Baoyi Shao: current Ph.D. student at the School of Biological Science and Medical Engineering at Southeast University, in Nanjing, China. Her research interests are mainly focus on optical biosensors for the detection of cancer markers, especially the analysis of exosomes.

Zhongdang Xiao: received his PhD degree from Southeast University in 1998. After worked as a post-doctoral fellow at University of Houston, he became a visiting professor at Harvard Medical School. Currently, Dr. Xiao is a full professor in the School of Biological Science and Medical Engineering at Southeast University and the deputy director of the National Key Laboratory of Bioelectronics. In recent years, his research direction is drug delivery based on exosomes, biosensors of cancer biomarkers and stem cell technology.