Trends in Biotechnology

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Review

Recent Advances in Biosensors for Detecting Cancer-Derived Exosomes

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Early detection and effective treatments are two of the greatest challenges in the fight against cancer. Cancer-derived exosomes are attractive biomarkers for the early diagnosis and therapeutic response evaluation of cancer. Here, we review recent advances in biosensors for the detection of cancer-derived exosomes. We discuss the potential exosomal biomarkers of various cancers, which can be applied as indicative targets in the design of biosensors. We further describe the fabrication of exosome detection biosensors with respect to biological recognition strategies and signal transduction techniques, which involve integrated scientific and technological aspects of analytical chemistry and nanotechnology. Furthermore, future research directions and challenges in using cancer-derived exosomes for point-of-care (POC) testing are presented.

Highlights

Exosomes are ideal candidates for reliable next-generation biomarkers for use in noninvasive methodologies for the early diagnosis and therapeutic response evaluation of cancer.

With the development of biosensors for the detection of exosomes, individuals in resource-limited regions who previously had no access to advanced diagnostic tools can benefit and cancer therapy can also be improved, creating a promising trend for POC usage.

The most recent advances in biosensors used to detect cancer-derived exosomes have focused on target choice, biological recognition strategies, and signal transduction techniques.

Although various biosensors have been reported and summarized for the detection of cancer-derived exosomes, the challenges and some of the crucial issues in this field still need to be addressed before their use in clinical applications.

Exosomes: A New Type of Cancer Biomarker

Cancers kill millions of people every year, most of whom are from developing countries. **Early detection** (see Glossary) and effective treatments are two of the greatest challenges in the fight against cancer [1,2]. However, traditional diagnostic methods for cancers are primarily based on endoscopy, computed tomography, X-rays, positron emission tomography, magnetic resonance imaging, and invasive tissue biopsies [3]. These methods are neither accessible to large populations nor practical for repeated screenings. **Cancer biomarkers** are emerging as one of the most promising strategies for cancer diagnostics and therapeutics. A cancer biomarker is a 'molecular signature' that can provide accurate information on the staging and the mechanisms underlying the initiation of cancer [4,5]. Although the number of potential biomarkers identified for different types of cancer is rapidly increasing, there is still a large gap between biomarker studies and their clinical usage because of many challenges, such as low concentration in human body fluids and poor stability of those potential biomarkers [6].

Cancer-derived exosomes, which typically have sizes of 30–150 nm and densities of 1.13–1.19 g/ml, are excreted into bodily fluids (such as serum, plasma, and urine) with high abundance and stability [7]. The exosomes play critical roles in tumorigenesis and cancer progression, including immunosuppression, angiogenesis, cell migration, and invasion, and more importantly, carry information regarding the tumor microenvironment [8]. Based on these features, exosomes are ideal candidates to be used as reliable next-generation biomarkers for noninvasive early cancer diagnosis and evaluation of therapeutic efficacy. On January 21, 2016, the first exosome-based cancer diagnostic product was marketed in the USA [9,10]. Although their clinical utility is still in its infancy, analyzing cancer-derived exosomes for monitoring cancer onset, evaluating therapy response, and prognosis has attracted significant attention.

Biosensors: A Trend Toward Detecting POC Exosomes

Although recent research in the field of exosomes has received widespread attention, there is still a lack of effective quantitative methods for exosome detection. There is an urgent need to identify and validate exosomes in body fluids in a rapid and cost-effective manner. In recent years, in an

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attempt to achieve sensitive exosome detections, **point-of-care (POC)** assays, especially **biosensors**, have attracted great attention from scientists and technicians worldwide [11–15]. The superior properties of biosensors to instrument-dependent technologies include their affordability, ease of use, rapid response, high sensitivity, excellent specificity, and multiplexing capability [16]. With the development of biosensors for the detection of exosomes, individuals in resource-limited regions who previously had no access to advanced diagnostic tools can benefit, and cancer therapy can also be improved [17]. Therefore, it is conceivable that the use of exosome biosensors will increase significantly in various POC clinical settings. Some previous reviews [7,18–22] have summarized exosome isolation and analysis methods, but not from a specialized biosensing perspective for diagnosis and prognosis. We will review recent advances of exosome-detecting biosensors associated with detailed target choice, biological recognition strategies, and signal transduction techniques (Table 1, Key Table). These techniques show a promising trend toward POC usage.

Biosensor Target Choice

Exosomes are composed of a characteristic lipid bilayer with an average thickness of ~5 nm [55]. The lipid bilayer is enriched in cholesterol, phosphatidylserine, and ceramide. Constitutive membranes are composed of **tetraspanins**, **adhesion molecules**, **proteases**, and **transmembrane receptors** according to the donor cell membranes [56]. Cancer-associated antigens are highly enriched on the surface of cancer-derived exosomes [57]. Therefore, exploiting the differences of their surface composition is the most promising approach for the simple and rapid identification of exosomes. For the development of biosensors, tetraspanins (such as CD9, CD63, CD81, and CD82) or lipid rafts (such as cholesterol, phosphatidylserine, and ceramide) on the surface of exosomes are typically employed as the targets for total exosome detection [58]. For cancer-associated antigens on the surface of exosomes, a vast array of different proteins can be detected, depending on the host cell (such as CEA, EpCAM, HER2, IGFR, LMP1, MUC18, and PSMA), which are used as diagnostic and therapeutic indicators for a variety of cancers [59]. Some exosomal biomarkers that have been used as the targets in the design of currently available biosensors are summarized in Table 2.

Biological Recognition in Biosensors

The choice of the **biological recognition element** depends entirely on the binding affinity and efficiency between the recognition element and target. The most classic biological recognition is the 'lock and key' model of antigen–**antibody** interaction [60,61]. Exosomal proteins are efficacious antigens and corresponding antigen–antibody immunoreactions have been well established in the development of biosensors [23–25,27,28,35,36,38–40,42–44,46,47,49–52]. In recent years, **aptamers** have been utilized as alternatives to antibodies as novel biological recognition molecules. Because of their cost-effectiveness, high stability, and strong resistance, many aptamers have been used to develop biosensors [26,29,30,32,33,45,53,54]. Interestingly, the aptamer sequences can be split into two different fragments to capture exosomes [31]. In addition, **peptides** are also employed as specific recognition elements that provide high performance with respect to binding affinity, scalability, quality, platform versatility, and cost-effectiveness, with the help of the protein evolution strategies. For example, the exosome capture of LXY30 and Vn96 can be used to quantitate exosomes efficiently in biosensors [62,63].

Special structural compositions on exosomes also provide opportunities for identification. Lipid anchors, such as cholesterol and oleyl ether anchors, can insert into the lipid bilayers of exosome membranes though hydrophobic interactions. Lipid anchors are a new biorecognition choice with excellent efficiency that is not affected by the amount of proteins on the surface of exosomes and have been used in the development of many biosensors [28,32,36]. In addition to biological recognition, biophysical interactions can also be considered during biosensor development.

Glossary

Adhesion molecules: mediated molecules involved in the development of adhesion bonds, which play a main role in several pathologies such as cancer and inflammatory diseases.
Antibodies: large Y-shaped proteins that are typically produced by mammalian immune systems or phage display technology and used to identify invading organisms or substances.
Aptamers: short oligonucleotides with 30–40 nucleobases that are synthesized *in vitro* without the need for animal or cell cultures and have the ability to bind specific analytes.
Biological recognition element: core part of biosensors due to their role in recognizing the targets of interest using biological molecules.
Biosensors: analytical devices that incorporate a biological material, a biologically derived material, or a biomimic that is intimately associated with or integrated within a physicochemical transducer or transducing microsystem.
Cancer biomarkers: indicators of a biological state or condition in biological fluids, such as blood and urine, which can be a protein, a protein fragment, DNA/RNA, or an organic chemical produced by cancer cells.
Early detection: detecting cancers at their earliest stages is necessary for improving the survival rate of cancer patients.
Lateral flow biosensor (LFB): a paper-based device consisting of four components: a sample pad, a conjugate pad, a nitrocellulose membrane-containing test and control lines, and an absorbent pad.
Limit of detection (LoD): the lowest concentration of a target that can be detected at a specified level of confidence.
Peptides: short chains of approximately 50 or fewer amino acid monomers linked by peptide (amide) bonds, which are distinguished from proteins on the basis of their small size.
Point-of-care (POC): patient care in the emergency room, in primary clinics, at home, or in other nonhospital settings, where diagnoses can be made and treatment regimens can be considered.
Proteases: enzymes that help proteolysis, which localize on the exosome surface secreted by various cell types.

For instance, self-assembled gold nanoislands exhibit higher specificity toward exosomes than other extracellular vesicles (EVs) due to their special membrane properties [41]. Coordination chemistry was introduced in recognition systems, most recently for detecting cancer-derived exosomes, which used zirconium-phosphate coordination chemistry via the electrostatic interaction between the Zr^{4+} ion and phospholipid membranes of exosomes [34]. In most cases, the recognition strategy of a biosensor is a combination of these various factors because exosomes carry multiple recognition sites, which favors the building of sandwich structures.

Signal Transduction in Biosensors

This section gives a typical and recapitulative description of the **signal transduction element** with respect to optical and electrochemical (EC) signals (Figure 1).

Colorimetric Biosensors

Colorimetric biosensors used to detect exosomes are of great importance for practical POC applications due to their easy operation and convenient readout. Typically, colorimetric biosensors can be easily and instantly observed with the naked eye through a color change that yields a 'yes/no' answer or semiquantitative result without any additional analytical instrumentation. Colorimetric biosensors can be divided into two formats: paper-based and solution-based.

Paper-based colorimetric biosensors generally use paper as the substrate and are favorable for on-site application owing to their ease of use and small sample volume needed. Recently, **lateral flow biosensors (LFBs)** have been reported; these are considered to be one of the most promising new technologies owing to their simplicity, rapid analysis, low costs, and high sensitivity and specificity. In most cases, LFBs consist of four components: a sample pad, a conjugate pad, a nitrocellulose membrane containing test and control lines, and an absorbent pad (Figure 1A). Basically, the sample solution migrates by capillary action and is passed over all of these pads. The targeting exosomes are captured by the capture element fixed on the test line and the detection elements labeled with nanoparticles are then attached on the exosomes to form sandwich complexes, which results in the color change that can be visualized due to the accumulation of nanoparticles on the test line. Oliveira-Rodríguez and colleagues first demonstrated a colorimetric LFB for the detection of exosomes from the human melanoma cell line Ma-Mel-86c [23]. They used a mixture of anti-CD9 and anti-CD81 (1:1) as capture antibodies and anti-CD63 labeled with gold nanoparticles as a detection antibody. The detection can be completed in 15 min, with a **limit of detection (LoD)** of 8.5×10^5 exosomes/ml. Furthermore, the same research group expanded the types of the used nanoparticles from gold nanoparticles to carbon black and to magnetic nanoparticles (Figure 1B) [24]. López-Cobo and colleagues explored different exosomal antigens for LFB development, showing that using exosomal MICA and CD9 as targets was more reasonable [25].

Solution-based colorimetric biosensors that use nanomaterials or enzymes as signal tags to generate color change can be advantageous for rapid and simple detection using relatively large sample volumes. Typically, solution-based biosensors employ gold nanoparticles that display red color as the colorimetric signal. Jiang and colleagues reported a biosensor platform that uses aptamers to protect gold nanoparticles from aggregating in a high-salt solution (Figure 1C) [26]. In the presence of exosomes, the specific binding between the exosomal protein and the corresponding aptamer occurred, resulting in color change of the solution due to the aggregation of gold nanoparticles (Figure 1D). Liu and colleagues demonstrated a highly sensitive biosensor called proximity ligation assay-recombinase polymerase amplification-transcription-mediated amplification assay, which is based on triggering gold nanoparticle aggregation with a concomitant color change from red to purple [27]. Another type of widely used solution-based biosensor is based on blue color signals generated by horseradish peroxidase (HRP)-

Signal transduction element: core part of biosensors function as physicochemical transducers or transducing microsystems, which can be optical, electrochemical, thermometric, magnetic, or micromechanical in nature.
Surface-enhanced Raman scattering (SERS): a surface-sensitive technique that enhances Raman scattering by molecules adsorbed on rough metal surfaces or by nanostructures.
Surface plasmon resonance (SPR): a label-free, real-time technique that allows molecular interactions occurring on the top of a gold layer to be detected based on monitoring changes in the refractive index.
Tetraspanins: a family of four-transmembrane proteins, which could make a major contribution in exosomal message transfer and selective target binding.
Transmembrane receptors: integrins mediating cell–cell or cell–extracellular matrix adhesion, such as integrins β1 and β2.

Key Table

Table 1. Summary of Biosensors for the Detection of Exosomes

Type	Substrate	Target	Recognition element	Signal element	Signal amplification	Detection limit	Sample	Refs
Colorimetric biosensors	Paper-based lateral flow biosensor	Exosomal CD9, CD81, and CD63	Antibody	Gold nanoparticles	NA	8.5×10^5 exosomes/μl	Melanoma (Ma-Mel-86c) cells and human plasma	[23]
	Paper-based lateral flow biosensor	Exosomal CD9, CD81, and CD63	Antibody	Gold nanoparticles, carbon black, and magnetic nanoparticles	NA	3.4×10^6 extracellular vesicles/μl	Human plasma	[24]
	Paper-based lateral flow biosensor	Exosomal MICA and CD9	Antibody	Gold nanoparticles	NA	5×10^7 exosomes/μl	Melanoma (Ma-Mel-55 and Ma-Mel-86c) cells and human serum	[25]
	Solution-based biosensor	Exosomal CD63, EpCAM, PDGF, PSMA, and PTK7	Aptamer	Gold nanoparticles	NA	NA	Cervical cancer (Hela) cell, prostate cancer (PC-3), acute lymphoblastic leukemia (Ramos), and acute lymphoblastic leukemia (CEM) cells	[26]
	Solution-based biosensor	Exosomal latent membrane protein 1 (LMP1) and epidermal growth factor receptor (EGFR)	Antibody	Gold nanoparticles	Proximity ligation assay (PLA)-recombinase polymerase amplification (RPA)-transcription-mediated amplification (TMA)	10^2 particles/ml	Nasopharyngeal carcinoma (NPC) cells and human plasma	[27]
	Solution-based biosensor	Exosomal CD9 and cholesterol	Antibody and cholesterol anchor	HRP-H_2O_2-TMB	Hybridization chain reaction (HCR)	2.2×10^3 exosomes/μl	Hepatocellular carcinoma (HepG2) cells	[28]
	Solution-based biosensor	Exosomal CD63	Aptamer	Nanozyme-H_2O_2-TMB, single-walled carbon nanotubes (s-SWCNTS)	NA	5.2×10^5 particles/μl	Breast tumor (MCF-7) cells and human serum	[29]
	Solution-based biosensor	Exosomal CD63	Aptamer	Nanozyme-H_2O_2-TMB, graphitic carbon nitride nanosheets (g-C3N4 NSs)	NA	13.52×10^5 particles/μl	Breast tumor (MCF-7) cells and human serum	[30]
Fluorescent biosensors	Paper-based biosensor	Exosomal CD63	Split aptamer	Upconversion nanoparticles (UCNPs) and gold nanorods (AuNRs)	NA	1.1×10^3 particles/μl	Hepatocellular carcinoma (HepG2) cells	[31]

	Solution-based biosensor	Exosomal CD63 and cholesterol	Aptamer and cholesterol anchor	Copper nanoparticles (CuNPs)	Copper-mediated signal amplification strategy	4.8×10^4 particles/μl	Hepatocellular carcinoma (HepG2) cells and human serum	[32]
	Solution-based biosensor	Exosomal CD63 and EpCAM	Aptamer	Cy3 and FAM fluorophores	DNase I enzyme digested cycle	2.1×10^4 particles/μl	Colorectal cancer (SW480) cells and human serum	[33]
	Solution-based biosensor	Exosomal CD63 and phospholipid bilayer	Antibody and zirconium-phosphate coordination chemistry	Calcein	NA	7.6×10^3 particles/μl	Cervical cancer (Hela) cell	[34]
	Microfluidic-based biosensor (Y-shaped)	Exosomal CD9, CD63, CD81, and EpCAM	Antibody	β-galactosidase (SβG) and di-β-D-galactopyranoside (FDG) system	NA	50 particles/μl (80 aM)	Ovarian cancer (COLO-1) cells and human plasma	[35]
	Microchip-based biosensor (digital output)	Exosomal glypican 1 (GPC-1) and membrane	Antibody and poly (ethylene glycol) oleyl ether anchor	Fluorescein isothiocyanate isomer (FITC), 5-carboxyfluorescein (FAM), and 6-carboxy-Xrhodamine (ROX) fluorophores	Rapid isothermal nucleic acid detection assay (RIDA)	Single-particle level	HeLa and Panc-1 cells and human urine	[36]
Surface plasmon resonance biosensors	Gold chip-based biosensor	Exosomal HER2	Antibody	NA	NA	8.28×10^3 exosomes/μl	Human serum	[37]
	Gold chip-based biosensor	Exosomal CD9, CD63, CD82, CD41b, E-cadherin, EpCAM	Antibody	NA	NA	4.87×10^7 exosomes/cm^2	Human hepatocellular carcinoma (MHCC97H and MHCC97L) cells	[38]
	Gold chip-based biosensor	Exosomal EpCAM, CD24, CA19-9, CLDN3, CA125, MUC18, EGFR, HER2, CD63	Antibody	NA	Star-shaped Au nanoparticles	3000 exosomes (670 aM)	Ovarian cancer (CaOV3) cells	[39]
	Gold chip-based biosensor	Exosomal CD9, CD171, CD81, and GM1	Antibody	NA	NA	1 μg/ml	Human plasma	[40]
	Gold nanoislands-based biosensor	Exosome	Biophysical interaction	NA	NA	0.194 μg/ml	Lung cancer (A-549 cells, SH-SY5Y cells), mouse blood serum, and mouse urine	[41]
Surface-enhanced Raman scattering biosensors	Solid-based biosensor	Exosomal CD9, CD63, MIF, GPC1, EGFR, and EpCAM	Antibody	Au@Ag nanoparticles @*p*ATP	NA	1 particles/2μl (9×10^{-19} mol/l)	PANC-01 and HPDE6-C7 cells and human serum	[42]

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Table 1. (continued)

Type	Substrate	Target	Recognition element	Signal element	Signal amplification	Detection limit	Sample	Refs
	Solid-based biosensor	Exosomal EpCAM, CD44, HER2, EGFR, IGFR, CD81, CD63, and CD9	Antibody and electrostatic interactions	Gold nanorods@QSY21	NA	2×10^6 exosomes/ml	Breast cancer (MDA-MB-231, MDA-MB-468, and SKBR3) cells and human plasma	[43]
	Solution-based biosensor	Exosomal CD63 and HER2	Antibody	Au@Ag nanorods @DTNB@SiO2	NA	1200 exosomes (268 aM)	Breast cancer (SKBR3) and normal human embryonic lung fibroblasts (MRC5) cells	[44]
	Solution-based biosensor	Exosomal CD63, CEA, HER2, and PSMA	Aptamer	Gold nanoparticles@ DTNB, gold nanoparticles @MMC, and gold nanoparticles @2NAT	NA	32 exosomes/μl, 73 exosomes/μl, 203 exosomes/μl	Breast cancer cells (SKBR3), prostate cancer cells (LNCaP), colorectal cancer cells (T84), human serum, and human blood	[45]
Electrochemical biosensors	Conventional electrode-based biosensor (carbon electrode)	Exosomal CD63, CD9, HER2, and FAM134B	Antibody	CdSe quantum dots (CdSeQDs)	NA	100 exosomes/μl	Breast cancer (BT-474), colon cancer (SW-48) cell, and human serum	[46]
	Conventional electrode-based biosensor (gold electrode)	Exosomal CD81 and syntenin	Antibody	Ferricyanide-ferrocyanide redox couple	NA	1.9×10^5 particles/ml (320 aM)	Human embryonic kidney 293 (HEK283) cells	[47]
	Conventional electrode-based	Exosomal CD63 and EpCAM	Aptamer	Ti_3C_2 MXenes nanosheets	NA	125 particles/μl	Melanoma cells line (B16), breast cancer cell line (MCF-7), human liver cancer	[48]

	biosensor (gold/glassy carbon electrode)						cell line (HepG2), and human serum	
	Screen-printed electrode-based biosensor	Exosomal CD63, EpCAM, CD24, CA125	Antibody	HRP-H_2O_2-TMB	NA	3×10^4 exosomes	OV90, OVCAR3, OCVA420, and TIOSE6 cells and human plasma	[49]
	Screen-printed electrode-based biosensor	Exosomal CD63 and CD3	Antibody	HRP-H_2O_2-TMB	NA	1.6×10^4 particles	Jurkat T cells and human urine	[50]
	Screen-printed electrode-based biosensor	Exosomal CD9	Antibody	HRP-H_2O_2-TMB	NA	2×10^2 particles/μl	Breast tumor (MCF-7) cells and human serum	[51]
	Screen-printed electrode-based biosensor	Exosomal CD9 and human epidermal growth factor receptor 2 (HER2)	Antibody	Ferricyanide--ferrocyanide redox couple	NA	4.7×10^5 exosomes/μl	BT-474 cells and human serum	[52]
	Screen-printed electrode-based biosensor	Exosomal EpCAM	Aptamer	Ferricyanide-ferrocyanide redox couple	Nanotetrahedron (NTH)	2.09×10^4 particles/ml	Hepatocellular carcinoma (HepG2) cells	[53]
	Micropatterned electrode-based biosensor	Exosomal CD63	Aptamer	Methylene blue (MB)	NA	1×10^6 particles/ml	Hepatocellular carcinoma (HepG2) cells	[54]

catalyzed H_2O_2-oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) system. He and colleagues used immunomagnetic beads with an anti-CD9 antibody to capture exosomes from HepG2 cells [28]. Subsequently, a bivalent-cholesterol (BChol)-labeled DNA anchor was inserted into the exosome membrane that could promote a HRP-linked hybridization chain reaction for blue color generation. A sensitivity of 2.2×10^3 exosomes per microliter was obtained, which was 100-fold higher compared with that of a conventional ELISA. However, using a natural enzyme as the signal tag leads to many disadvantages of HRP, such as requiring complicated purification process and cautious storage conditions. Recently, various nanozymes with intrinsic properties of mimicking natural enzyme activities have gained extensive attention for biosensor construction because of their advantages such as low cost, bulk preparation capability, and high resistance to pH and temperature changes. For example, single-walled carbon nanotubes (s-SWCNTs) (Figure 1E) were reported as nanozymes and integrated CD63 aptamers for the 'turn-off' detection of exosomes [29]. Similarly, graphitic carbon nitride nanosheets (g-C3N4 NSs) were also used to develop a solution-based biosensor that showed a clearly observable color change from dark blue to light blue with an increasing concentration of the CD63-positive exosomes (Figure 1F) [30]. Therefore, such simple methods hold great potential of becoming a routine tool for quantitative detection of exosomes.

Fluorescent Biosensors

Fluorescent biosensors developed for detecting exosomes show great advantages because of their high sensitivity. The generation of a fluorescent signal typically depends on the use of fluorescent dyes, fluorophores, fluorescent proteins, or fluorescent nanoparticles. Fluorescent biosensors can be divided into three system formats: paper-based, solution-based, and microplatform-based.

Paper-based fluorescent biosensors can also be designed as a simple analytical platform, namely, paper-based analytical devices (PADs) (Figure 1G). Chen and colleagues reported on the development of a fluorescent PAD using upconversion nanoparticles (UCNPs) and gold nanorods (AuNRs) as a fluorescence-quenching nanopair to detect exosomes derived from hepatocellular carcinoma (HepG2) cells (Figure 1H) [31]. Interestingly, the CD63 aptamer sequence was split into two different fragments [capture probe (CP) and detection probe (DP)] to capture exosomes and form a sandwich structure. UCNPs and CP were preloaded onto the sodium periodate-oxidized filter paper to generate green luminescence. AuNRs modified by DP were mixed with exosomes and aliquoted onto the filter paper such that the formed sandwich structure reduced the distance between AuNRs and UCNPs, which quenched the green luminescence through luminescence resonance energy transfer. The authors read the luminescence intensities of the colored paper images to quantify the exosome concentration. This proof of concept provides a novel strategy for developing split-based biosensors in the field of clinical exosome detection.

Solution-based fluorescent biosensors are generally based on the formation or release of fluorescent nanoparticles or fluorophores. A simple and cost-effective fluorescent biosensor for exosome detection was developed based on fluorescent copper nanoparticles (CuNPs) (Figure 1I) [32]. Specifically, the sandwich structure was built by the reactions among aptamer-modified copper oxide nanoparticles (CuO NPs), exosomes, and cholesterol-modified magnetic beads (MBs) (Figure 1J). Fluorescence was achieved through the acidolysis of transforming CuO NP into copper (II) ions (Cu^{2+}) and the reduction of Cu^{2+} into fluorescent CuNPs by sodium ascorbate in the presence of poly(thymine). Another example of a solution-based fluorescent biosensor is based on the release of fluorophores [33]. This biosensor uses fluorophore-labeled aptamers that are quenched by graphene oxide (GO) and then released in the present of exosomes. The detection cycle in their design is achieved by a DNase I enzyme; when it digests the aptamers and releases the fluorophores, the available exosomes enter a new detection cycle to generate

Table 2. Summary of Exosomal Biomarkers Currently Used as Targets for Biosensors

Type	Biomarker	Description	Refs
Exosome biomarkers	CD9	An abundant and characteristic membrane tetraspanin protein present in exosomes.	[23–25,28,35,38,40,42,43,46,51,52]
	CD63	An abundant and characteristic membrane tetraspanin protein present in exosomes.	[23,24,26,29–33,35,38,39,42–46,49,50,54]
	CD81	An abundant and characteristic membrane tetraspanin present in exosomes.	[23,24,35,40,43,47]
	CD82	An abundant and characteristic membrane tetraspanin protein present in exosomes.	[38]
	Cholesterol	A waxy, fat-like substance present in the characteristic lipid bilayer of exosomes.	[28,32]
Cancer-associated antigens	CA19-9	Cancer antigen 19-9, a carbohydrate tumor-associated antigen present in a wide range of malignant conditions, including ovarian carcinomas.	[39]
	CA125	Cancer antigen 125, also known as mucin 16, is a member of the mucin family glycoprotein and is the most frequently used biomarker for ovarian cancer detection.	[39,49]
	CD3	A potent biomarker used to detect T cell-derived exosomes. Cancer-associated antigens of exosomes can suppress T cell signaling molecules and induce apoptosis.	[50]
	CD24	A small, heavily glycosylated cell adhesion molecule expressed in hematological malignancies and a variety of solid tumors, such as ovarian cancer.	[39,49]
	CD41b	Platelet glycoprotein IIb, CD41b, can be used as biomarker for prostate cancer.	[38]
	CD44	A transmembrane glycoprotein that plays a role in cancer cell migration and matrix adhesion in response to a cellular microenvironment. The most widely used biomarker for breast cancer and colorectal cancer detection.	[43]
	CD171	The L1 adhesion molecule (CD171) represents a strongly neuronal biomarker for a variety of solid tumors, such as ovarian cancer and endometrial carcinomas.	[40]
	CLDN3	Claudin 3 (CLDN3) is a transmembrane protein crucial in the formation and function of tight junctions that exhibits elevated expression in ovarian cancer.	[39]
	CEA	Carcinoembryonic antigen (CEA) is overexpressed in a variety of solid tumors, such as colorectal, breast, stomach, and lung cancer.	[45]
	E-cadherin	Epithelial cell–cell adhesion molecule (E-cadherin) is used as biomarker for a variety of solid tumors, such as breast, prostate, and gastric cancer.	[38]
	EGFR	Epidermal growth factor receptor (EGFR) is a cell-surface receptor whose overexpression and mutations are associated with many cancers, such as breast and lung cancer and spongioblastoma.	[27,39,42,43]
	EpCAM	Epithelial cell adhesion molecule (EpCAM) is transmembrane glycoprotein expressed exclusively in epithelial and epithelial-derived neoplasms, that is viewed as an important biomarker for a variety of cancers, such as lung, breast, colorectal, and prostate cancer.	[26,33,35,38,39,42,43,49,53]
	FAM134B	Family with sequence similarity 134-member B (FAM134B), also known as mucin JK1, is used as biomarker for colon cancer.	[46]
	GM1	Ganglioside GM1 favors the aggregation of Aβ in Alzheimer's disease.	[40]
	GPC1	Glypican 1 (GPC1) serves as biomarker of pancreatic cancer.	[42]
	HER2	Human epidermal growth factor receptor 2, also known as receptor tyrosine kinase erbB-2, whose overexpression plays a major role in the development and progression of multiple cancers, such as breast and stomach cancer.	[39,43–46,52]

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Table 2. (continued)

Type	Biomarker	Description	Refs
	IGFR	Insulin-like growth factor receptor (IGFR), an important growth factor system involved in the development of the organism, is associated with many cancers, such as non-small cell lung, breast, prostate, and colon cancer.	[43]
	LMP1	Latent membrane protein 1 (LMP1) is an integral membrane protein important in B cell immortalization by Epstein-Barr virus that is associated with many cancers, such as lung and nasopharyngeal cancer.	[27]
	MICA	Major histocompatibility complex class I-related chain A (MICA) is a highly polymorphic functional cell-surface protein associated with many cancers, such as cervical cancer and melanoma.	[25]
	MIF	Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine and mediator of acute and chronic inflammatory diseases and is overexpressed in various tumors, such as gastric, pancreatic, and breast cancer.	[42]
	MUC18	Mucin 18 (MUC18) is a cell-surface glycoprotein and cell adhesion molecule whose expression is a prognostic marker in epithelial ovarian cancer.	[39]
	PDGF	Platelet-derived growth factor (PDGF) is a crucial regulator of mesenchymal cell migration and proliferation that is an important biomarker for many cancers, such as small cell lung, breast, and colon cancer.	[26]
	PSMA	Prostate-specific membrane antigen (PSMA) is a type II integral membrane glycoprotein that is an important biomarker for prostate cancer.	[26,45]
	PTK7	Protein tyrosine kinase-7 (PTK7), is a catalytically inactive receptor tyrosine kinase that is upregulated in many cancers, including colon, lung, and gastric cancer and acute myeloid leukemia.	[26]

an enhanced fluorescence signal. Wang and colleagues proposed an exosome-zirconium-liposome sandwich structure to detect exosomes by using zirconium-phosphate coordination chemistry [34]. This principle was based on the strong electrostatic interaction between the Zr^{4+} ion and phospholipid membranes of exosomes and calcein-packaged liposomes with easy operation, no modification probe, and sensitive performance.

Microplatform-based fluorescent biosensors can be powerful for exosome detection because they can improve analytical performance via device fabrication and can even provide digital output by random distribution. Zhang and colleagues reported a Y-shaped microfluidic devise based on a new GO/polydopamine (PDA) nanointerface for exosome analysis [35]. Such Y-shape configuration can effectively enhance the capture efficiency through the microchannel and simultaneously suppress nonspecific exosome adsorption. As a result, the ultrasensitive assay was developed with a very low detection limit of 50 particles/μl, satisfying the requirements for basic and clinical investigations. To further increase the sensitivity, Tian and colleagues presented a digital exosome detection microchip integrated with nucleic acid-based amplification (Figure 1K) [36]. The digital output of '1' indicates the presence of an exosome in a chamber, while a chamber without an exosome was recorded as '0'. After random distribution of samples, digital signals can perform quantitative analysis at a single-particle level by counting the number of positive chambers. In addition to the innovative output method, this design also introduced a rapid isothermal nucleic acid detection assay for thousands of dimensional magnifications. Biocompatible anchor molecule DNA and hybridized DNA probe-fluorescein isothiocyanate were used to display total exosomes (Figure 1L, green), while specific antibody–DNA conjugates

and hybridized DNA probe–carboxyrhodamine were used to display GPC-1(+) exosomes (Figure 1L, red).

Surface Plasmon Resonance Biosensors

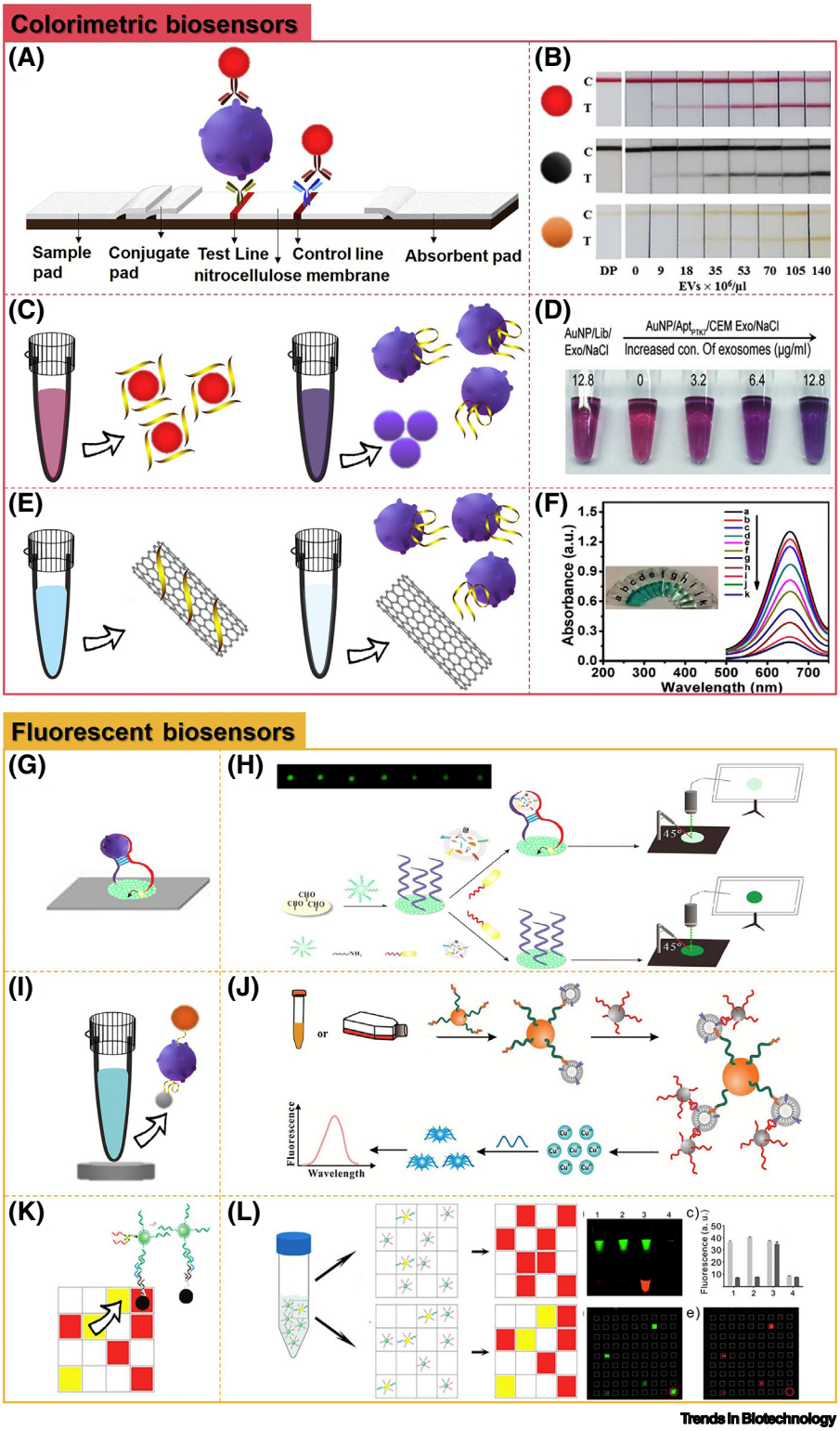
Surface plasmon resonance (SPR) is a label-free, real-time analysis technique that can detect molecular interactions on the surface of a gold layer by monitoring the changes in its refractive index. SPR is extremely sensitive to the biological binding events occurring within 200 nm (wave depth) of the gold layer. Such distance closely matches the dimension of exosomes. Therefore, SPR-based biosensors are perfectly suited for the study of exosomes.

The substrate used for reported SPR biosensors for exosome detection has been primarily based on gold chips (Figure 2A). There are slight differences in designs, including the use of one-step or two-step detection strategies and the use of a spectrometer setup with a microscope or imaging system. The one-step SPR biosensors have only one layer of antibody-modified gold layer [37]. After the injection of the exosome sample, real-time signal changes can be recorded due to the binding between the antibody and the exosome (Figure 2B). For instance, Zhu and colleagues utilized antibodies specific to exosomal membrane proteins (CD9, CD63, and CD82) and tumor-related transmembrane proteins (CD41b, EpCAM, and E-cadherin) to print each spot of the gold microarrays (Figure 2C) [38]. The two-step SPR biosensors typically injected additional components onto a gold chip to enhance the signal or explore more detection information. In a study by Im and colleagues, the gold surface of 12 microfluidic channels was prefunctionalized by a layer of polyethylene glycol and corresponding antibodies [39]. After being combined with exosomes, the biosensor was subjected to secondary labeling with nanoprobes. For instance, gold nanostars (Figure 2D) were used for signal enhancement with the help of a biotin-neutravidin linker system, resulting in an enhancement of 300% (Figure 2E). In addition, the measurement of the SPR signal using a spectrometer and an imager were also compared in Im's study and showed an excellent agreement [39]. Picciolini and colleagues also designed a two-step SPR biosensor to detect the multiple exosome subpopulations of neural origin directly in human plasma [40]. The purpose of their second injection step was to enhance the signal as well as to quantify the amount of membrane components of exosomes in each subpopulation. Thus, secondary antibodies (anti-CD81 and anti-ganglioside GM1, and AbCam) were injected into the system (Figure 2F) to generate an additional SPR signal and demonstrate that exosomal proteins are not expressed homogeneously but exhibit variable abundance according to the exosome cellular origin (Figure 2G). In addition to a regular gold chip, random arrays of self-assembly gold nanoislands (SAM-AuNIs) chip with sensitive and low-cost properties were introduced for exosome detection without the need for any additional chemicals (Figure 2H). Interestingly, in the work reported by Thakur and colleagues [41], a SAM-AuNIs chip without any antibody functionalization could generate a localized SPR response to exosomes instead of other EVs; this was based on a higher specific biophysical interaction between the exosome membrane and the chip (Figure 2I). Therefore, the biophysical detection of exosomes can be performed directly using the bare antibody-unfunctionalized SPR biosensor, which has been confirmed using SH-SY5Y and A-549 cells and mouse blood serum (Figure 2J).

Surface-Enhanced Raman Scattering Biosensors

Surface-enhanced Raman scattering (SERS)-based biosensors have been used for exosome detection because of their remarkable enhanced analytical signals, including single molecule-level sensitivity and insensitivity to quenching. SERS biosensors can be divided into two system formats: solid-based and solution-based.

Solid-based SERS biosensors primarily consist of two parts: the solid substrate with the recognition element and the SERS nanoprobe with recognition element, which can form sandwich



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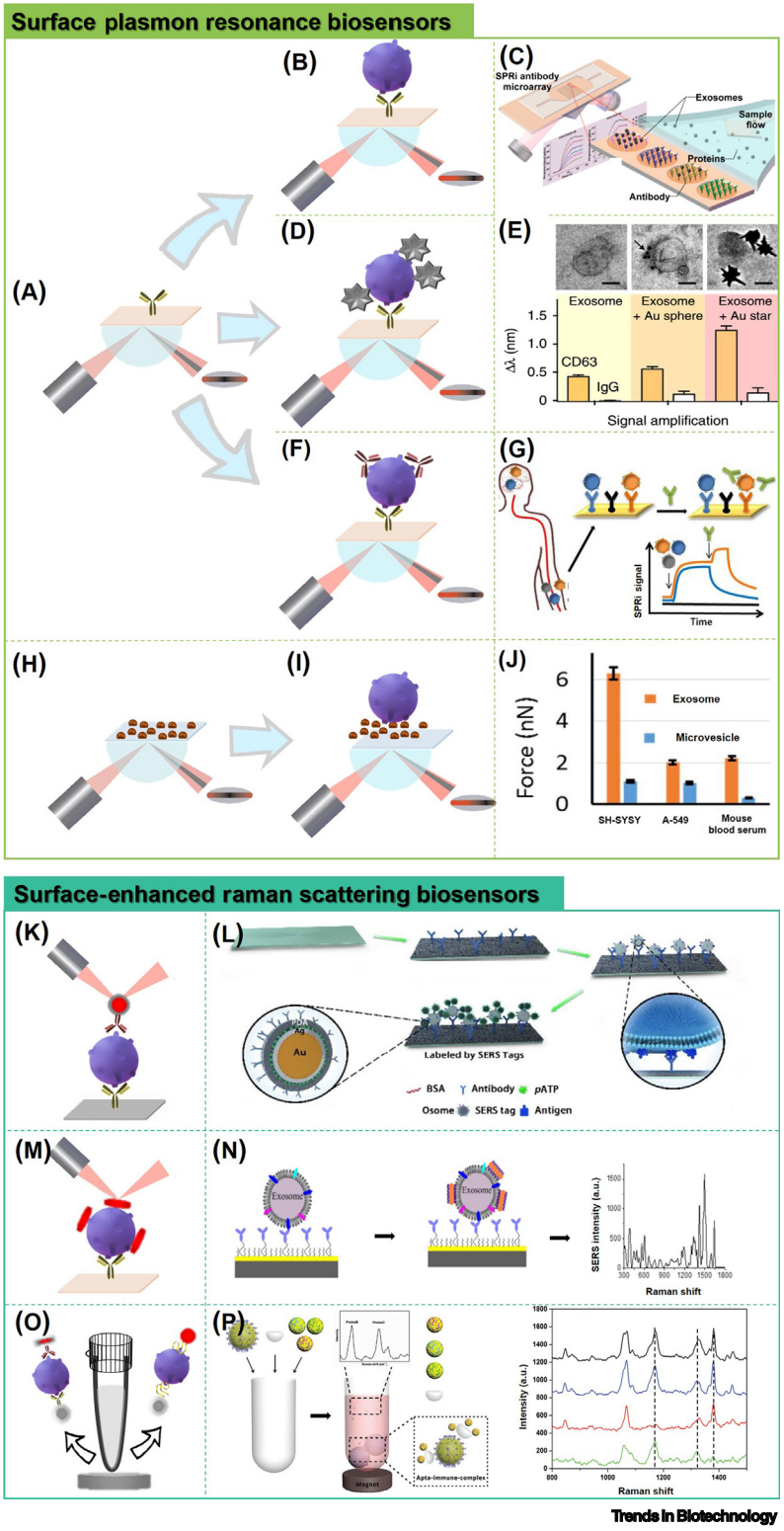
structures with exosomes. The glass slide substrate used was a standard type used in early studies (Figure 2K). In recent studies, the substrate of the SERS biosensor has been improved by being coated the with a rough nanoscale metal layer, which can form SERS 'hot spots' to enhance the sensitivity (Figure 2M). In addition, the SERS nanoprobes employed in the biosensors are also critical to the performance of the biosensors. The SERS nanoprobe primarily consists of three parts: a metal core with various shapes and sizes, a Raman reporter, and a recognition element. For instance, Li and colleagues developed a SERS nanoprobe using self-polymerization reaction. This nanoprobe consists of a gold metallic core coated with silver, a Raman reporter molecule *p*ATP, and an antibody as the recognition element (Figure 2L) [42]. In their SERS biosensor design, the glass slide substrate was modified by PDA to provide enough space for capturing antibodies. The assay had a detection limit of only one exosome in 2 μl of sample solution (approximately 9×10^{-19} mol/l) and was able to distinguish various stages of tumor node metastasis. Kwizera and colleagues changed the shape of the gold metallic core and fabricated a SERS nanoprobe based on such nanoparticles (Figure 2N) [43]. In their design, AuNR coated with the organic dye QSY21 were loaded on the glass substrate coated with gold to detect exosomes with a portable Raman spectrometer (TSI ProRaman spectrometer). The AuNRs and exosomes were bound through electrostatic adsorption due to the negatively charged exosomes (zeta potential around –10 mV) and positively charged AuNRs (zeta potential around +35 mV). Instead of locking a specific protein, this nonspecific binding can increase binding efficiency and improve sensitivity.

Solution-based SERS biosensors typically use nanoparticles with magnetic properties as substrates because they can separate the exosome-linked sandwich structure from complex samples by applying an external magnetic field to form more 'hot spots' (Figure 2O). In addition, the magnetic nanoparticle-substrate has a larger surface area than the glass slide-substrate, which can capture exosomes directly in the solution with higher efficiency and improved sensitivity. For instance, Zong and colleagues synthesized magnetic nanobead substrate by coating Fe_3O_4 nanoparticles with a silica shell and antibodies. Using such nanoparticles as the substrate and Au@Ag nanorods@DTNB@SiO_2@antibodies as the SERS nanoprobe, exosomes were detected by forming a sandwich complex [44]. In their study, tumor-derived exosomes were successfully detected in tumor cells and normal cells with an LoD of 268 aM. Instead of antibodies, Wang and colleagues modified aptamers as recognition elements [45]. They coated different aptamers and different Raman reporters onto gold nanoparticles as SERS nanoprobes, while magnetic nanobeads@SiO_2@Au@CD63-aptamer were prepared as the capturing substrates (Figure 2P). The aptamers and Raman reporters were matched to form three different probes that can be used to distinguish the species of exosomes in one step, simulating cancer screening [45].

Electrochemical Biosensors

EC biosensors can convert the recognition of a biomolecule into electrical signals, which include current, potential, and impedance. There are numerous labeled tags that are designed to be applied in EC biosensors for signal generation, including enzyme, ferrocenes, interactive

Figure 1. Examples of Colorimetric and Fluorescent Biosensors for the Detection of Exosomes. (A) Scheme of the lateral flow biosensor (LFB). (B) Examples of LFBs using gold nanoparticles, carbon black, and magnetic nanoparticles as labels (image reproduced with permission from [24]). (C,D) Scheme and an example (image reproduced with permission from [26]) of the solution-based biosensors using the red color of gold nanoparticles as an output. (E,F) Scheme and an example (image reproduced with permission from [30]) of the solution-based biosensors using the blue color of nanozymes as an output. (G,H) Scheme and an example (image reproduced with permission from [31]) of paper-based fluorescent biosensors using upconversion nanoparticles and gold nanorods as a fluorescence-quenching nanopair to detect exosomes. (I,J) Scheme and an example (image reproduced with permission from [32]) of solution-based fluorescent biosensors based on the formation of fluorescent copper nanoparticles. (K,L) Scheme and an example (image reproduced with permission from [36]) of microplatform-based fluorescent biosensors for digital detection of exosomes at the single-nanoparticle level. Abbreviations: Apt, aptamer; AuNP, gold nanoparticle; CEM Exo, human acute lymphoblastic leukemia exosome; EV, extracellular vesicles; Lib, library DNA.



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electroactive substances, or nanoparticles. With the development of integrated circuit technology and the emergence of new types of electrodes, EC biosensors are gradually developing in the direction of miniaturization and portability. Therefore, EC biosensors can be divided into three system formats: conventional, screen-printed, and micropatterned electrode-based.

Conventional electrode-based EC biosensors for exosome detection primarily use glassy carbon electrodes or gold electrodes (Figure 3A). Boriachek and colleagues used bare glassy carbon disk electrodes as substrates and dissolved CdSe quantum dots (CdSeQDs) as the EC signal tags (Figure 3B) [46]. MBs modified by a generic tetraspanin antibody (CD9 or CD63) were used as the first layer and CdSeQDs functionalized-biotinylated tumor-specific antibodies were used as the third layer. In the presence of the target, a sandwich can be formed to detect exosomes from breast and colon cancer cell lines and serum samples. Finally, retained CdSeQDs were dissolved in HNO_3, such that the resulting peak current was associated with the stripping of Cd^{2+} and the number of cancer-specific exosomes. This method enabled the highly sensitive detection of 100 exosomes/μl, which may allow the system to be used for the detection of several other tumor-specific exosomes. Gold electrodes are another commonly used candidate substrate because of their high sensitivity, rapid response rate, and fast mass transfer rate. Li and colleagues used a gold electrode as a substrate and ferricyanide-ferrocyanide redox couple as an EC signal tag to quantify both external (tetraspanin) and internal (syntenin) exosome-specific markers [47]. A gold/glassy carbon electrode was employed by Zhang and colleagues [48], and aptamer-modified MXenes nanosheets were used as nanoprobe catalysts for the luminol to enhance the electrogenerated chemiluminescence (ECL) intensity. This strategy combines the EC and luminescent techniques together with excellent performances.

Screen-printed electrode-based EC biosensors have attracted significant attention in recent years because of their low cost, disposability, and design flexibility compared with traditional electrode-based EC biosensors (Figure 3C). Jeong and colleagues combined magnetic enrichment, antibody recognition, and enzymatic signal amplification with a screen-printed electrode for high-throughput measurements [49]. They also designed a portable, eight-channel device that was used to screen EVs in plasma samples from ovarian cancer patients (Figure 3D). Similarly, Park and colleagues implemented the same design to capture T cell-derived CD3-positive exosomes in urine to detect kidney transplant rejection [50]. Instead of using MB enrichment, Doldán and colleagues directly used their functional screen-printed electrode to test serum samples with different dilutions [51]. The exosomal tetraspanin biomarker (CD9) was chosen as the only protein target, and two formats of 'signal-off' and 'signal-on' were developed to optimize analytical performance. Yadav and colleagues further assayed a breast cancer-specific biomarker (human epidermal growth factor receptor 2, HER2) as a biomarker to identify breast cancer-derived exosomes and used corresponding antibodies to form a sandwich format [52]. To further

Figure 2. Examples of Surface Plasmon Resonance (SPR) and Surface-Enhanced Raman Scattering (SERS) Biosensors for the Detection of Exosomes. (A) Scheme of an SPR biosensor for the modification of a layer of antibodies on a gold chip surface. (B,C) Scheme and an example (image reproduced with permission from [38]) of a one-step gold chip-based SPR biosensor for the binding of injected exosomes. (D,E) Scheme and an example (image reproduced with permission from [39]) of a two-step gold chip-based SPR biosensor for secondary labeling gold nanostars. (F,G) Scheme and an example (image reproduced with permission from [40]) of a two-step gold chip-based SPR biosensor for the secondary injection of antibodies. (H,I) Scheme of an SPR biosensor for the fabrication and injected exosomes binding of a random array of self-assembly gold nanoislands chip. (J) An example of a self-assembly gold nanoislands chip-based SPR biosensor for its application in SH-SY5Y and A-549 cells and mouse blood serum (image reproduced with permission from [41]). (K,L) Scheme and an example (image reproduced with permission from [42]) of solid-based SERS biosensors using a glass slide as substrate. (M,N) Scheme and an example (image reproduced with permission from [43]) of solid-based SERS biosensors. (O,P) Scheme and an example (image reproduced with permission from [45]) of solution-based SERS biosensors. Abbreviations: BSA, bovine serum albumin; PDA, polydopamine; SPRi, surface plasmon resonance imaging.

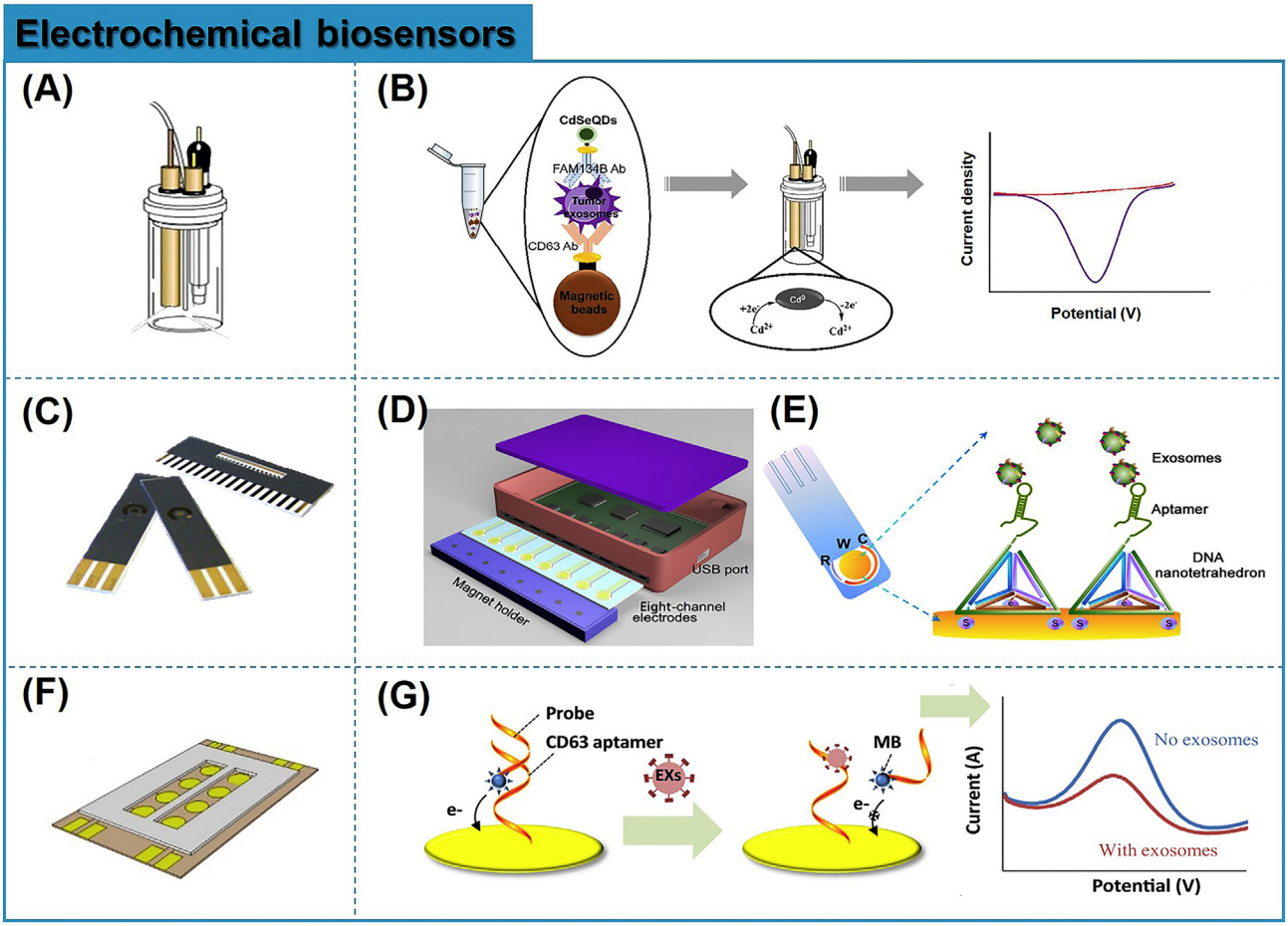

Figure 3. Examples of Electrochemical (EC) Biosensors for the Detection of Exosomes. (A,B) Scheme and an example (image reproduced with permission from [46]) of a conventional electrode (glassy carbon electrode)-based EC biosensor using dissolved CdSe quantum dots (CdSeQDs) as an EC signal. (C) Scheme of screen-printed electrodes. (D) An example (image reproduced with permission from [49]) of a screen-printed electrode-based EC biosensor coupled with an eight-channel device. (E) An example (image reproduced with permission from [53]) of competitive format screen-printed electrode-based EC aptasensor with DNA nanotetrahedron enhancement. (F,G) Scheme and an example (image reproduced with permission from [54]) of a micropatterned electrode-based EC biosensor with competitive format. Abbreviations: EX, exosome; MB, magnetic bead.

improve the sensitivity of the direct detection, Wang and colleagues developed a DNA nanotetrahedron (NTH)-assisted aptasensor exhibiting a 100-fold increase in sensitivity compared with traditional aptamer-based biosensors (Figure 3E) [53]. The principle of NTH enhancement allows for the maintenance of spatial orientation and reduces the hindrance effect for better biomolecular recognition.

Micropatterned electrode-based EC biosensors have been miniaturized to detect exosomes using a small sample volume (Figure 3F). Zhou and colleagues immobilized aptamers onto micropatterned electrode surfaces and prelabeled probing strands with redox moieties (methylene blue or MB) to hybridize aptamers [54]. In the presence of exosomes, probing strands with redox reporters were released, leading to a reduced EC signal (Figure 3G). This method represented a 100-fold decrease in the LoD compared with commercial immunoassays, which can be further applied as a powerful tool for clinical exosome studies.

Concluding Remarks and Future Perspectives

We have reviewed the most recent advances in biosensors for detecting cancer-derived exosomes with respect to target choice, biological recognition strategies, and signal transduction techniques. These studies show that the biosensor-based detection of exosomes is becoming a promising alternative to conventional techniques, as it offers advantages, such as reduced detection time and low-cost, in POC diagnosis and prognosis. A general overview of the advantages, limitations, and potential breakthrough strategies of the various biosensing approaches for exosome detection have been summarized in Table 3. Although various biosensors have been reported for the detection of cancer-derived exosomes, the challenges and some of the crucial issues in this field still need to be addressed before their use in clinical applications.

(i) Specificity and multiplexing abilities: it is important to validate the specific exosome markers according to the different requirements for early cancer diagnosis or therapeutic response evaluation. For instance, circulating exosome PD-L1 was shown to be a novel marker to reflect distinct states of antitumor immunity. [64] Moreover, if there is not an ideal single exosome marker with a certain diagnostic attribute, multiplex exosome markers need to be detected simultaneously in a single biosensor or arrays.

(ii) Sensitivity of biosensors: only highly sensitive biosensors can detect predictive exosomal markers in early cancer stages to have prognostic value and guide further therapy. Therefore, it is important to quantify the concentration of exosomal markers in serum, plasma, or urine at a very low detection limit. Successful novel nanomaterials, such as nanoflowers [65,66] and nanozymes [67,68], that can be easily designed, synthesized, and linked with exosomes for signal cascade or amplification, are highly desirable.

(iii) Stability of nanoparticles: in most cases, the generated or enhanced signal in biosensors is directly linked to the stability of the nanoparticles. However, signals of biosensors will vary due to poor reproducibility of synthesized nanoparticles without accurately controlled conditions. Further studies are required in the fabrication of biosensors, such as how to reduce batch

Outstanding Questions

What exosome markers or rational combinations will dominate future POC applications?

How can we solve crossreaction problems for multiplex exosome marker detection in a single biosensor or arrays simultaneously?

What are the meaningful concentration ranges for various exosome markers in different cancer stages as well as therapy response degrees?

How can we reduce interference of nanoparticles and guarantee robust performance of the cost-effective biosensors for different clinical samples?

How can we integrate all units into one user-friendly device to allow reliable and selective stratification of patients toward successful clinical outcomes?

The first exosome-based cancer diagnostic product was marketed in the USA on January 21, 2016. What will be the medical industry response to various biosensors for exosome detection in the future?

Table 3. A Critical Comparison and Assessment of Various Exosome-Detecting Biosensors

Type	Advantage	Limitation	Potential breakthrough strategy
Colorimetric biosensors	Easy-to-use detection Rapid response Naked-eye detection Cost-effective system	Low sensitivity Limited multiplexing capability Limited quantification capability	Preconcentration of exosomes Fabrication of platforms Integration of smartphone or glucometer
Fluorescent biosensors	High sensitivity Flexible fluorescence-quenching capability Multiplexing capability	Costly equipment with both excitation light and fluorescence measurement Fluorescent label may impair binding	Cost-effective and user-friendly device development Interfacial engineering investigation
Surface plasmon resonance biosensors	High sensitivity Real-time detection Label-free system	Bulky equipment Interferences Complex system	Portable and user-friendly device development Systematic optimization Integration of microfluidic system
Surface-enhanced Raman scattering biosensors	Superior sensitivity Multiplexing capability Complex biological samples applicability	Costly equipment Fabrication of the SERS nanoprobe Difficulty of data analysis	Cost-effective and user-friendly device development Stable and modifiable Raman reporter usage Intelligent software analysis
Electrochemical biosensors	High sensitivity Rapid response Cost-effective system Multiplexing capability Easily miniaturized devices	Surface functionalization challenging Sample matrix effects Reproducibility problems	Interfacial engineering investigation Preconcentration of exosomes Stable electrode and nanoparticles

variation and enhance stability of nanoparticles, how to introduce standards of exosomes, and how to set up control tests of biosensors to improve stability.

(iv) User-friendly devices: for POC uses, scientists need to avoid expensive equipment to make biosensors portable and easy to use. For instance, smartphone-, pH meter-, or glucometer-integrated biosensors are promising options. Therefore, there is an urgent need to involve advanced manufacturing technology (e.g., 3D printing) [69] into the fabrication of biosensors to produce simple and robust systems that are user-friendly in settings outside of the laboratory. Overcoming each of these challenges will bring a revolutionary impact on the development of exosome-detecting biosensors. Additionally, some questions still need to be addressed before their use in clinical applications (see Outstanding Questions). We believe that sensitive, accurate, inexpensive, convenient, multiplexed, efficient, and fully integrated biosensor systems for exosome detection will play increasingly important roles in the area of global health in the near future.

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