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Aptamer-based biosensor for detecting carcinoembryonic antigen

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ABSTRACT

Carcinoembryonic antigen (CEA), as one of the common tumor markers, is a human glycoprotein involved in cell adhesion and is expressed during human fetal development. Since the birth of human, CEA expression is largely inhibited, with only low levels in the plasma of healthy adults. Generally, CEA will overexpressed in many cancers, including gastric, breast, ovarian, lung, and pancreatic cancers, especially colorectal cancer. As one of the important tumor markers, the detection of CEA has great significance in differential diagnosis, condition monitoring and therapeutic evaluation of diseases. Conventional CEA testing typically uses immunoassay methods. However, immunoassay methods require complex and expensive instruments and professional personnel to operate. Moreover, radioactive element may cause certain damage to the human body, which limits their wide application. In the past few years, biosensors, especially aptamer-based biosensors, have attracted extensive attention due to their high sensitivity, good selectivity, high accuracy, fast response and low cost. This review briefly classifies and describes the advance in optical and electrochemical aptamer biosensors for CEA detection, also explains and compares their advantages and disadvantages.

1. Introduction

Tumor markers have great practical value in tumor screening, diagnosis and efficacy evaluation [1–4]. Tumor markers are considered to be substances that can be attributed to the development of normal cells or carcinogenesis at different stages of cell development [5]. It is most commonly proteins and sugar lipids, but also includes DNA, RNA and microRNA (miRNA) [6–9]. Since its first description in 1965, carcinoembryonic antigen (CEA) is the most thoroughly studied tumor marker [10].

CEA is a human glycoprotein that is involved in cell adhesion and expressed during human fetal development. It is produced by normal fetal intestinal tissue and epithelial tumors, and its serum level can also be increased in non-malignant diseases such as inflammatory bowel disease [11,12]. Following birth, CEA expression has been largely inhibited, with very low plasma CEA levels in healthy adults [13]. However, CEA is abnormally expressed in many human cancers, such as gastric cancer, breast cancer, ovarian cancer, lung cancer, and pancreatic cancer, especially colorectal cancer (CRC) [10,14,15]. It is abundantly expressed in approximately 95% of CRC, and some studies have extensively demonstrated the association between serum CEA (s-CEA) levels and CRC [16]. So, detecting CEA has great significance in differential diagnosis, condition monitoring and therapeutic evaluation of diseases [17–20]. In recent years, sensor analysis and detection of CEA has attracted widespread attention.

Most of reported medical practices for CEA detection was based on immunoassay techniques, including enzyme-linked immunosorbent assays (ELISAs) [21,22], electrochemical immunoassays [23], radioimmunoassay (RIA) and immunoradiometric assay (IRMA) [24], fluorescence immunoassay [25] etc.. Although immunoassay has good selectivity, most developed immunoassays rely on specific markers, such as fluorescent molecules, radioactive elements or enzymes, in order to translate the binding information of antigen-antibody pairs into readable signals [26]. In addition, immunoassay methods require complex and expensive instruments that require professional operating, and radioactive elements can cause damage to body, thus limiting its wide application [27–29]. Therefore, developing rapid, high-sensitivity, high-selectivity, cost-effective methods for detecting CEA is critical for the detection of human diseases.

With the development of biosensor technology, biosensor has attracted great attention as a tool for analysis and diagnosis. It is an evolving field that meets the needs of people for fast, simple, selective and low-cost analysis. Biosensor is a three-part analysis device: a biosensing (or biorecognition) component, a transducer, and a signal

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processing unit [30,31]. A key part of the biosensor is the transducer, which takes advantage of the physicochemical changes that accompany the reaction [31]. Biosensors can be divided into electrochemical, optical, piezoelectric, magnetoelectric, etc. By their signal transduction methods [32,33]. Optical and electrochemical biosensors are two widely used biosensing platforms [34,35].

In the last few decades of research, aptamer-based biosensors have been applied to CEA detection due to their good sensitivity and selectivity, high accuracy, fast response, and low cost [36–38]. An aptamer is a single-stranded RNA or DNA molecule selected in vitro from the nucleic acid molecular library by systematic evolution of ligands by exponential enrichment (SELEX) to specifically combine targets with high affinity (nucleic acids, small molecules, proteins, etc.) [39–41]. It can be considered functional analogs of antibodies and has a wide range of charge and structure combinations for a wide variety of biomedical, diagnostic, in vitro or in vivo biological imagings and therapeutic applications [42]. Due to the limitations of immunosensors, they are not suitable for a wide range of applications in detection. For example, some very small molecules cannot react and antibodies are unstable in extreme environments. The aptamer is flexible, repeatable, easy to fix and regenerate, no difference between batches, which has been widely used in the sensor field [43–48]. Since aptamers exhibited many beneficial aspects, aptamer-based biosensor systems have been developed to date for analysis of various classes of detectors.

This review summarizes the application of aptamers in CEA biosensors, and provides a detailed introduction in two aspects: aptamer-based optical biosensors and aptamer-based electrochemical biosensors.

2. Aptamer-based optical CEA biosensor

To date, optical biosensors are used in food safety, life sciences, environmental monitoring and medical testing, and have made great progress [49–51]. Optical biosensor includes transducers that can capture signals generated by the interactions of biometric element with target analyte and convert them into optical signals [52,53]. It is widely used in CEA biosensors due to its high specificity and sensitivity, direct and rapid quantification, easy miniaturization, and label-free detection [52,54]. This section highlights optical biosensor platforms that have been widely used to date, such as colorimetric, surface plasmon resonance, fluorescence, chemiluminescence and electrochemiluminescence biosensors. Table 1 summarizes the studies of optical biosensors used to determine CEA.

2.1. Colorimetric-based CEA aptamer biosensor

In aptamer-based optical technology, colorimetry has attracted great attention due to its low readout cost and visual inspection capabilities. The colorimetric biosensor is label-free which can be quickly observed color changes through the naked eye [77,78]. Colorimetric aptamer sensors mainly contain gold nanoparticles (AuNPs) sensors and HRP-mimicking DNAzyme sensors [79]. Due to their simple operation and portability without other measuring devices, it has the potential of commercial detection.

Gold nanoparticles (AuNPs) have many attractive properties, including unique optical properties and high molar extinction coefficients, which makes them sensitive probes for detecting biological analytes [80,81]. AuNPs are commonly used as colorimetric indicators owing to their strong distance-dependent optical properties [82]. However, colorimetric biosensor based on AuNPs still has some limitations. AuNPs have a tendency to non-specific aggregation in complex biological fluids. And the colorimetric biosensor requires AuNPs to have a uniform size and shape [78]. These problems require scholars to consider and explore in sensor design. Luo et al. developed a colorimetric biosensor for CEA detection based on a salt-induced aggregation of AuNPs and conformational changes in CEA single-stranded DNA aptamers. AuNPs were not stable in high concentrations of sodium chloride and aggregates, causing a color change from red to blue. A sensitive linear range and detection limit for CEA was received via adjusting the addition of the aptamer and NaCl, which were helpful and convenient to meet different detection needs. This method had a linear range between the CEA concentrations of 0–120 ng mL^{-1} with the limit of detection (LOD) 3 ng mL^{-1} [55].

Shahbazi et al. designed a simple colorimetric aptamer biosensor based on two unlabeled oligonucleotides, including CEA aptamer and split peroxidase-mimicking DNAzyme. In the absence of CEA, DNAzyme interacted with the aptamer, and there was no restriction on the formation of G-quadruplex, which could efficiently catalyzed H_2O_2-mediated oxidation of 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) with an output signal of colorimetric. Control experiments had shown that other existing proteins did not interfere with CEA signals. In addition, the aptamer biosensor in this strategy could detect CEA in saliva [57]. It is worth noting that the oxidation product of ABTS is unstable and rapidly decomposes into colorless in an aqueous medium. This problem needs to be solved [79].

2.2. Surface plasmon resonance-based CEA aptamer biosensor

Surface plasmon resonance (SPR) biosensor is attractive for biosensing applications because of the high sensitivity of the resulting evanescent field to local refractive index (RI) changes. SPR was first successfully used to build SPR-based biosensors to detect interactions of biomolecular in 1983 [83]. SPR is a strong electron-electron oscillation that can occur at the metal/dielectric interface [84]. This technology has attracted widespread attention due to its label-free, high selectivity, direct and real-time detection [85,86]. Because it is extremely sensitive to the refractive index of materials near the thin metal film, it is possible to detect the combination of biochemical molecules with high sensitivity on the surface in real time [87–89]. Direct detection of SPR is only applicable to analyte detection with molecular weights greater than 5000 Da, which is difficult to detect for low molecular weight analytes, but it can be improved by competitive and sandwich analysis [90]. At present, SPR has been widely used in the field of cancer detection [91,92]. Guo et al. reported a novel bifunctional electrochemical and SPR biosensor based on metal-organic framework (MOF) nanocomposites (Fig. 1). The composite material was embedded in a zirconium metal-organic framework (Zr-MOF, UiO-66) of silver nanoclusters (AgNCs) (AgNCs @ Apt @ UIO-66) using aptamers as templates. The synthesized AgNCs @ Apt @ UiO-66 had well biocompatibility and bioaffinity, and it was used as scaffold of SPR and electrochemical, which shown good selectivity, excellent stability and wide applicability in real human serum samples. This sensor showed a little higher LOD of 0.3 ng mL^{-1} at a concentration of 1.0–250 ng mL^{-1} [58].

2.3. Fluorescence-based CEA aptamer biosensor

Fluorescence is one of the most commonly used methods for optical sensors with versatility, simplicity, sensitivity, multi-analyte detection, non-destructive and reproducible [93,94]. By combining fluorescence technology into the sensing probe, the biometric process is converted to an optical signal because many targets and biosensing elements have no inherent spectral features [95]. Using fluorescence technology and aptamers, various aptamer-based fluorescence methods have been developed for CEA detection. According to the type of signal output, the design of fluorescent biosensor is mainly divided into two categories: turn-on and turn-off [96,97]. Turn-on and turn-off represent the enhancement and attenuation of the fluorescent signal, respectively. Signal changes can reflect the degree of the combining process, permitting quantitative measurement of analyte concentration.

2.3.1. Turn-off type of CEA biosensor

In the turn-off type, the binding of analyte to the aptamer results in

Table 1
Optical aptamer biosensors for CEA determination.

Method	Assay strategy	Nanomaterial	Donor, Exc/Em (nm)	Sensor detection buffer	Recognition motif	Linear range	LOD	Ref
Colorimetric	Salt-induced AuNPs aggregation	AuNPs	–	PBS	ssDNA	0–120 ng mL^{-1}	3 ng mL^{-1}	[55]
	Hyperbranched rolling circle amplification (HRCA)	AuNPs	–	Tris–HCl	ssDNA	5–500 pM	2 pM	[56]
	Label-free optical strategy	–	–	Tris	ssDNA	1–50 ng mL^{-1}	1 ng mL^{-1}	[57]
SPR	AgNCs @ Apt @ UiO-66 - SPR	AgNCs @ Apt @ UIO-66	–	PBS	ssDNA	1.0–250 ng mL^{-1}	0.3 ng mL^{-1}	[58]
Fluorescence	Visual fluorescence detection	CdTe/CdSe	CdTe/CdSe 365/550	PBS	dsDNA	0.05–20 ng mL^{-1}	6.7 pg mL^{-1}	[59]
	Surface-enhanced fluorescence (SEF)	AgNCs and AuNP	AgNCs 473/540	purified water	ssDNA	0.01–1 ng mL^{-1}	3 pg mL^{-1}	[60]
	Synthesize the blue-fluorescent carbon dots with tomato juice	CD	CD 367/440	PBS	ssDNA	1 ng mL^{-1} to 0.5 mg mL^{-1}	0.3 ng mL^{-1}	[61]
	"Mix-and-detect" procedure	–	PFN −/−	PBS	ssDNA	0.4–100 ng mL^{-1}	0.316 ng mL^{-1}	[62]
	Polymer point based FRET	PFO dots and AuNPs	PFO 380/440	HEPES	dsDNA	0.1–10 ng mL^{-1}	–	[63]
	FRET between upconversion nanoparticles (UCNPs) and graphene oxide (GO)	UCNP and GO	UCNP 980/500	HEPES	UCNPs ssDNA	0.03–6 ng mL^{-1}	7.9 pg mL^{-1}	[64]
	FRET between UCPs and palladium nanoparticles (PdNPs)	UCPs and PdNPs	UCPs 980/480	Tris-HCl	UCPs ssDNA	2–100 pg mL^{-1}	0.8 pg mL^{-1}	[65]
	The combination of GO and aptamer labeled QDs by CE.	CdSe/ZnS and GO	CdSe/ZnS –/634	PBS	QD ssDNA -	0.257–12.9 ng mL^{-1}	5 pg mL^{-1}	[66]
	The combined effects of FRET and internal filter effect (IFE)	CdTe-MSN and MoS_2 NSs	CdTe-MSN 365/590 and 731	PBS	ssDNA	0.001–10 pg mL^{-1}	0.7 fg mL^{-1}	[67]
	A four-color fluorescent nanoprobe for multiplexed detection and imaging	GO	Cy3 543/553	Tris-HCl	ssDNA	1.0–200 nM	0.62 nM	[68]
	Single oligonucleotide-mediated isothermal quadratic amplification (SOIQA)	GO	FAM 480/490	Tris-HCl	hairpin ssDNA	50 fg mL^{-1} to 50 ng mL^{-1}	28.5 fg mL^{-1}	[69]
	A cascade nucleic acid amplification strategy	–	NMM 399/610	Tris-HCl	dsDNA	0.001–2 ng mL^{-1}	0.3 pg mL^{-1}	[70]
	A smart DNA walker	–	NMM 399/610	Tris/Mg/K buffer	stem–loop structure DNA	10 pg mL^{-1} to 100 ng mL^{-1}	1.2 pg mL^{-1}	[71]
Chemiluminescence (CL)	Capillary electrophoresis-chemiluminescence (CE-CL) detection system	GO	–	PBS	HRP-ssDNA	0.0654–6.54 ng mL^{-1}	4.8 pg mL^{-1}	[72]
	All-in-one dual-aptasensor	–	–	PBS	CEA aptamer-linker-hemin aptamer	0–200 ng mL^{-1}	0.58 ng mL^{-1}	[38]
Electrochemiluminescence (ECL)	Surface-enhanced ECL	Ru @ SiO_2 -AuNPs	–	PBS	ssDNA	0.005–50 pg mL^{-1}	1.52 fg mL^{-1}	[73]
	Label-free ECL	ZnS–CdS/MoS_2	–	PBS	ssDNA	0.05–20 ng mL^{-1}	0.031 ng mL^{-1}	[74]
	Signal amplification	CdS-GR and AuNP	–	PBS	ssDNA	0.01–10.0 ng mL^{-1}	3.8 pg mL^{-1}	[75]
	paper-based bipolar electrode	PG-Fe_3O_4 NPs and AuNPs	–	PBS	ssDNA	0.1 pg mL^{-1} to 15 ng mL^{-1}	0.03 pg mL^{-1}	[76]

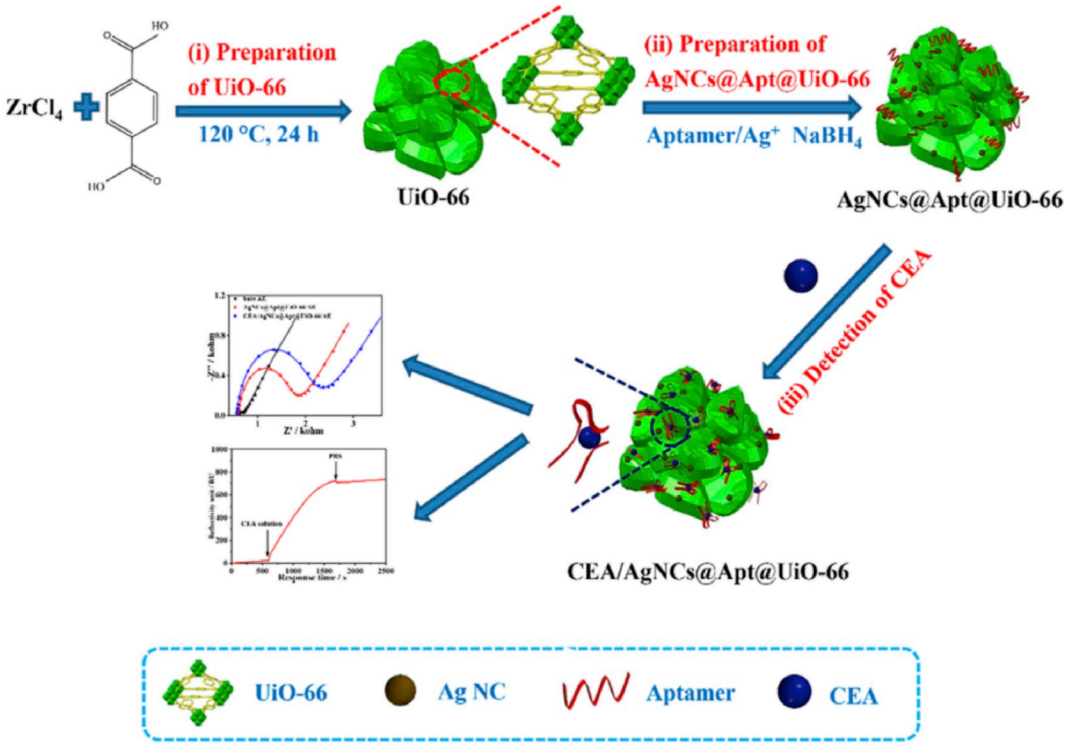

Fig. 1. Schematic diagram of CEA aptamer sensor using SPR technique based on the AgNCs@Apt@UiO-66 nanocomposite. Reprinted with permission from Ref. [58]. Copyright 2017 American Chemical Society.

a change in the structural conformation, causing the quencher to be in intimate contact with the fluorophore, thereby reducing fluorescence. The most important consideration in such scheme is the degree to which conformational changes of aptamers affect the distance between the quencher and fluorophore in the presence of targets [98]. Although biosensors based on turn-off type are generally not as sensitive as sensors based on the turn-on type, they can sometimes result in better targets detection by low-affinity aptamers. Importantly, simpler designs with fewer steps are required to "turn-off" biosensor that is both convenient and low-cost [99]. For example, Qiu et al. proposed an integrated paper-based analytical device (PAD) for visible fluorescence of CEA detection with CdTe/CdSe quantum dot (QD)-enzyme-impregnated paper (Fig. 2). Upon introduction of the CEA target in the detection cells, the target reacted specifically with an aptamer immobilized on a mesoporous silica nanocontainers (MSN), which opened the pore and resulted in glucose release. The immobilized glucose oxidase (GOD) oxidized the released glucose on paper to generate gluconic acid and hydrogen peroxide, which quenched the fluorescence of CdTe/CdSe QD [59]. However, the sensitivity of this method was relatively low due to the lack of enzyme amplification. Interestingly, based on complementary DNA hybridization, Yang et al. used AuNP to strengthen the fluorescence intensity of silver nanoclusters (AgNCs),

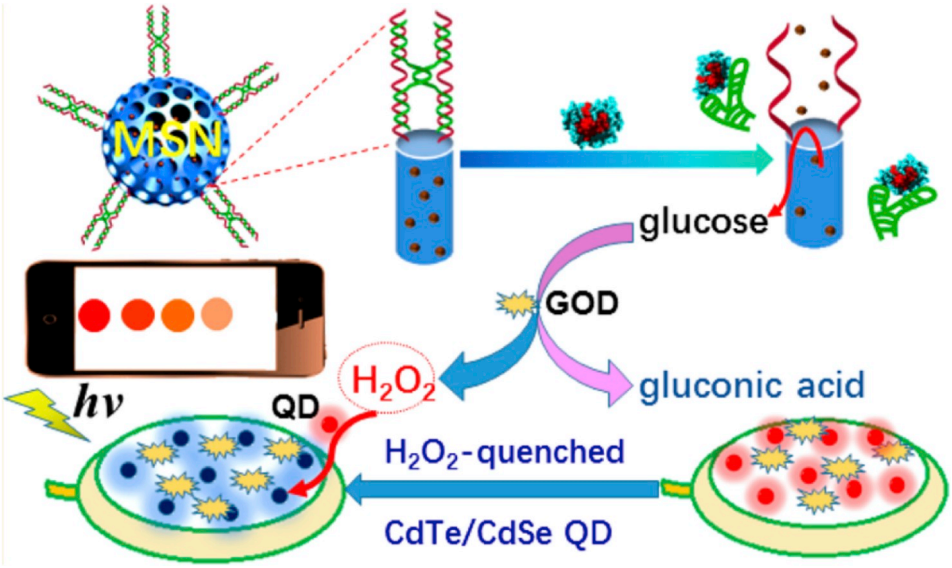

Fig. 2. A schematic diagram of a visually fluorescent integrated paper based analysis device (PAD) for CEA detection by coupling with a bioresponsive controlled-release system from DNA-gated mesoporous silica nanocontainers (MSNs). Reprinted with permission from Ref. [59]. Copyright 2017 American Chemical Society.

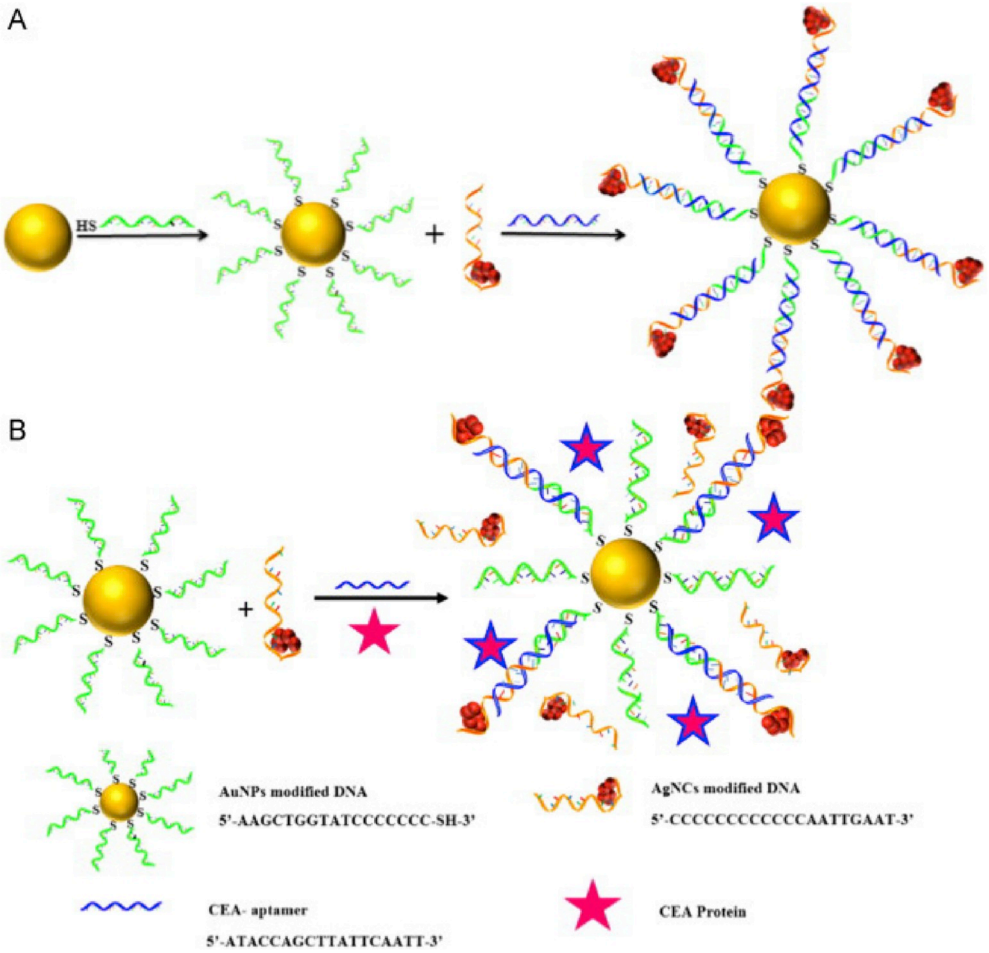

Fig. 3. A schematic diagram of CEA detection based on Surface-enhanced fluorescence (SEF): (A) schematic illustration of SEF occurring. (B) schematic illustration of the creative strategy for assaying CEA. Reprinted with permission from Ref. [60].

and successfully established a surface enhanced fluorescence (SEF) system to detect CEA (Fig. 3). AgNCs provided the original fluorescent signal, and DNA-modified AuNP was also used as a fluorescence enhancer. This method, which combined of nanoparticles and nanoclusters by virtue of aptamer, supplied a worthy model for sensitive detection of other trace targets in bioanalytical fields. The linear range was from 0.01 ng mL^{-1} to 1 ng mL^{-1} and LOD was down to 3 pg mL^{-1} [60].

2.3.2. *Turn-on type of CEA biosensor*

In general, fluorescence quenching, compared with the fluorescence opening method, caused an erroneous signal by accidental quenching of interfering substances. Therefore, turn-on type biosensors had the advantage of being easy to detect low concentration analytes and reducing false positive signals. Recently, Bao et al. designed a label-free biosensor using polyfluorene-based cationic conjugated polyelectrolytes (PFN^+) for the detection of two tumor markers (CEA and AFP). The aptamer effectively quenched the fluorescence of PFN^+ by forming a PFN^+-DNA complex. Aptamers formed a tighter and folded formation through specific protein-aptamer interactions, causing increased fluorescence intensity of PFN^+ when CEA presented. In addition, the sensor could quickly detect the target protein within 5 min without the need for complicated processing procedures and expensive instruments [62].

There are many methods for detecting CEA for turn-on type, which is mainly divided into two aspects: one is based on fluorescence resonance energy transfer (FRET), and the other is signal amplification technology.

2.3.2.1. FRET-based CEA biosensor. When fluorescence spectra of the donor molecules overlapped with the excitation spectra of receptor molecules, excitation of the donor fluorescent molecule induced fluorescence of the receptor molecule while the fluorescence intensity was decreased for the donor fluorescent molecule. This phenomenon is FRET. The extent of FRET is related to the distance between the donor and receptor molecules, which was typically 7–10 nm [100,101]. Xu et al. designed a four-color fluorescent nanoprobe for multiplex determination and imaging of tumor-related proteins in living cells. This was the first example of the coinstantaneous imaging of four intracellular protein biomarkers via nanoprobes. The fluorescence intensity was linear with CEA concentration from 1.0 nM to 200 nM, and the CEA detection limit was 0.62 nM [63]. However, traditional organic dyes have limitations such as narrow absorption, wide emission and photobleaching and the appearance of nanomaterials with fluorescent emission function solves these problems well [102–104].

The distinguishing feature of upconversion nanoparticles (UCNP) is the emission of shorter wave luminescence in near-infrared (NIR) radiation (anti-Stokes emission), where the inherent bioluminescent fluorophore cannot be excited and has no light scattering background [105–107]. Due to its near-infrared excitation and visible emission properties, the appearance of lanthanide-doped UCNP successfully decreased the background interference and significantly improved the signal-to-noise ratio [108,109]. Wang et al. reported a FRET aptamer biosensor using UCNP and graphene oxide (GO) for determination of CEA [64]. Similarly, the aptamer was modified with upconverting nanoparticles, but li et al. used palladium nanoparticles (PdNPs) as the quenching agent to construct the FRET system (Fig. 4). The interaction

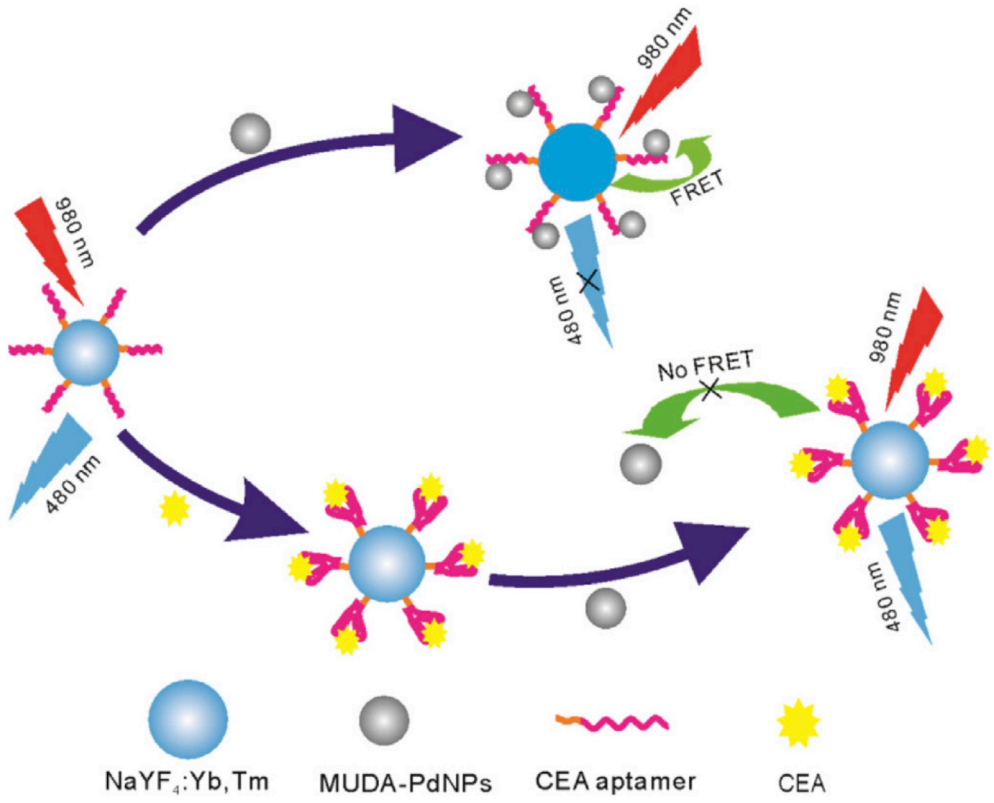

Fig. 4. Schematic diagram of FRET between UCP and palladium nanoparticles (PdNPs). Reprinted with permission from Ref. [65].

between suitable CEA and nitrogen functional groups of PdNPs brought the UCNP and PdNPs close together, resulting in a fluorescence quenching of 85% [65]. However, related studies have shown that water has a strong light absorption at 980 nm, resulting in a heating effect. This will cause 99% of the UC luminescence signal to be quenched due to non-radiative relaxation of the sensitizer and activator ions. This should be considered in the preparation of biosensors [105,110].

Quantum dots (QDs) are common fluorescence molecules that are generally used as fluorescent labeling for aptamers due to their broadband light absorption, low toxicity, resistance against photobleaching and strong photoluminescence emission [111,112]. Zhou et al. proposed a CEA content analysis strategy based on the combination of capillary electrophoresis (CE) and aptamer-labeled QD (Fig. 5). Combined with CE, the aptamer biosensor had advantages in dosage of samples and efficiency of separation was high. In this detection method, the fluorescence intensity had a linear relationship with CEA concentration from 0.257 to 12.9 ng mL^{-1}. Based on S/N = 3, the detection limit was approximately 5 pg mL^{-1} [66]. It is worth noting that the main problem of QDs is that the presence of cadmium in the core which has a certain toxicity, but this can be solved with an additional silicon shell coating [113].

Recently, another new type of fluorescent nanomaterial, polymer dots, has attracted wide attention due to the strong fluorescence emission caused by abundant p-electrons. Lin et al. introduced polymer dots into FRET-based biosensors to analyze specific proteins, while AuNPs were used as effective quenchers. CEA was quantitatively detected by changes in fluorescence intensity. There was a good linear range of CEA between 0.1 and 10 ng mL^{-1} [68].

2.3.2.2. Nucleic acid amplification technologies-based CEA biosensor. In order to improve sensitivity and enhance signal readout, signal amplification strategy that is based on nucleic acid amplification has been investigated and applied to CEA detection. Its purpose is to improve the performance of biosensors, resulting in higher sensitivity and a wider detection range. As a conventional amplification technique, although PCR has been widely used in various fields, the requirements of thermal cyclers have largely limited the use of PCR in resource-constrained environments and real-time measurements [114–116]. The development of isothermal amplification technology effectively avoids the shortcomings of PCR and enables amplification of targets at a constant temperature in a fast, simple and efficient manner, which is a promising alternative to PCR [117,118]. Xu et al. constructed a single oligonucleotide-mediated isothermal quadratic amplification (SOIQA) technology for FRET-based fluorescent aptamer biosensors. (Fig. 6). CEA bound to the aptamer and unfolded the aptamer hairpin probe labeled fluorophore to build a new DNA hairpin, resulting in catalytic recycling of the CEA and DNA sequence to carry out SOIQA. The large number of fluorophore-labeled single nucleotides produced by SOIQA were not adsorbed on GO, leading to a marked increase in the fluorescence intensity of target molecule amplification. This assay provided a low detection limit of 28.5 fg mL^{-1} for CEA concentration, ranging from 50 fg mL^{-1} to 50 ng mL^{-1}, with good specificity and a wide detection range of 6 orders of magnitude [69].

2.4. Chemiluminescence-based CEA aptamer biosensor

Chemiluminescence (CL) is an optical strategy that refers to the luminescence energy produced by a chemical reaction without using external light sources or optics. Compared to the fluorescence and other optical techniques, CL does not require an external light source and optical filters, avoiding the influence of stray light and the instability of light source [119–121]. In addition, the lifetime of the CL luminescent material is much longer than the fluorescent material [122]. However, due to the small CL reaction system and poor selectivity, the application of CL is limited. So it is often associated with separation technology to make up for its shortcomings. Zhou et al. designed a novel CEA determination method based on aptamer/graphene oxide (Apt/GO) combined with capillary electrophoresis-chemiluminescent (CE-CL).

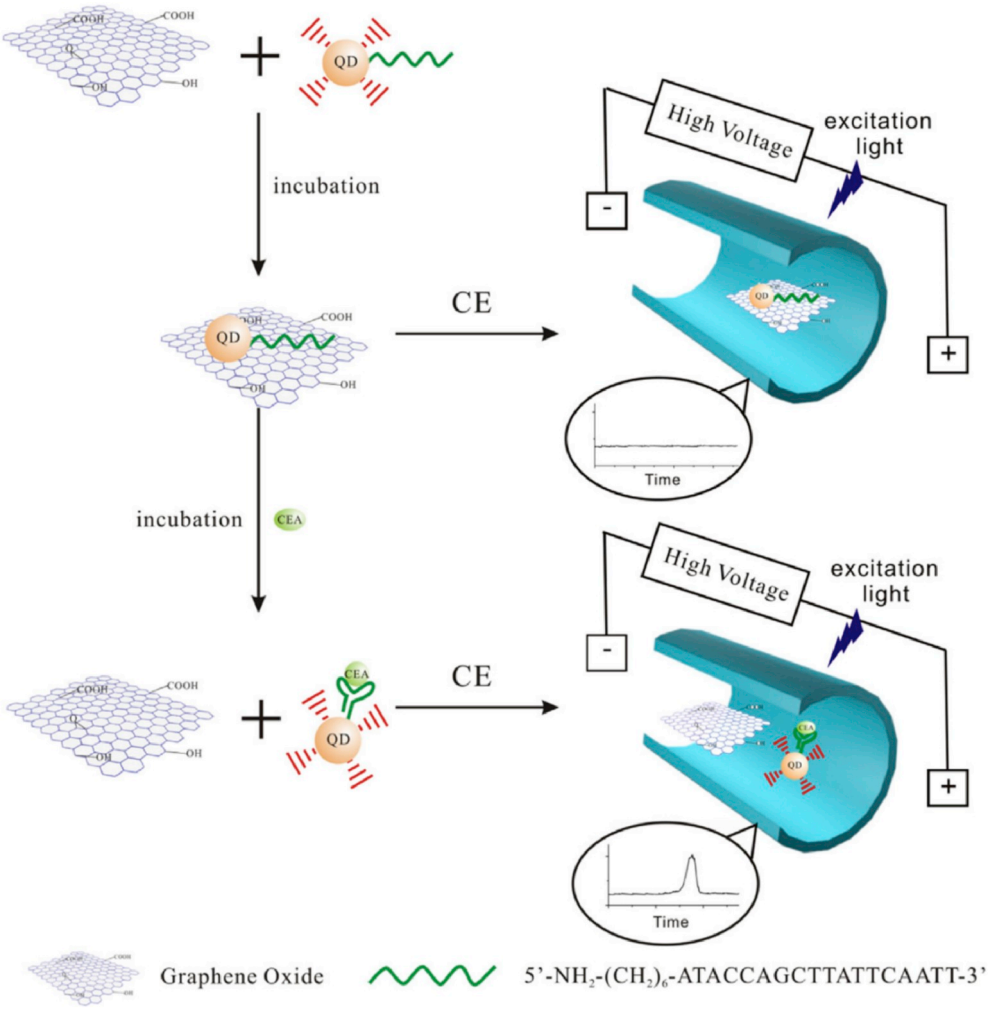

Fig. 5. Schematic diagram of CE combined with GO and aptamer-labeled QD for CEA detection. Reprinted with permission from Ref. [66].

When CEA existed, HRP-Apt-CEA complex was formed by HRP-Apt and CEA, leaving from GO, and then the CL signal catalyzed by this complex could be detected without any chemiluminescence resonance energy transfer (CRET). CL intensity was linear with the CEA concentration, ranging from 0.0654 to 6.54 ng mL^{-1} with a detection limit of approximately 4.8 pg mL^{-1} [72]. In another study, Khang et al. used a dual DNA aptamer (CEA aptamer combined with hemin aptamer) to develop a dual aptamer biosensor based on 1,1′-oxalyldiimidazole (ODI) CL detection. The detection of all-in-one aptamer biosensor can be completed in a short single incubation time without the need for time-consuming washing operations, thereby eliminating the waste and interference required by conventional ELISA [38].

2.5. Electrochemiluminescence-based CEA aptamer biosensor

Electrogenerated chemiluminescence (ECL) is the light produced by an electron transfer reaction between electrochemically generated reagents [123]. ECL combines the characteristics of electrochemical controllability and low chemiluminescence background and has advantage of cost-effective, simple optical setup and fast measurement, considering as a powerful analytical technique produced by electrochemical reactions [124,125]. Wang et al. designed a surface enhanced ECL (SEECL) biosensor for ultra-sensitive detection of target in human serum (Fig. 7). Researchers used Ru $(bpy)_3^{2+}$ doped SiO_2 nanoparticles ($Ru@SiO_2$) as ECL luminophores, and AuNPs acted as surface enhancement sources for ECL signal amplification. In the presence of CEA, $Ru@SiO_2$ and AuNP formed a network of nanostructures, which increased the ECL intensity by 30 times [73]. In another study, Wang et al. used ZnS–CdS nanoparticle (NP) modified molybdenum disulfide (MoS_2) nanocomposite electrodes as a sensing system and aptamers as identification molecules to prepare a sensitive ECL biosensor for CEA determination. The layered MoS_2 modified on the electrode greatly increased the surface area of the sensing interface, which largely increased the loading number of ZnS–CdS, generating more ECL signals. This strategy had a good application prospect and was successfully applied to the detection of CEA in human serum with a recovery rate of 80–111% [74].

3. Aptamer-based electrochemical CEA biosensor

Electrochemical biosensors are based on selective interactions between a target compound and a recognition element (enzyme, antibody, DNA/RNA, tissue or other biomolecules) to generate an electrical signal proportional to the concentration of the analyte compound. A typical electrochemical biosensor consists of three parts: working, reference and auxiliary electrodes [126,127]. A series of electrochemical techniques have been applied to biosensing applications, including impedimetric, amperometric, voltammetric, potentiometric, etc. [127–129].

One of the key advantages of electrochemical transduction compared to fluorescence analysis is that it does not contain labels and the conversion phenomenon is simple. Cheap electrodes can be integrated with simple electronic devices for fast detections in a compact, easy-to-use portable system. In general, electrochemical biosensors offer advantages such as high specificity of their biometric processes, low

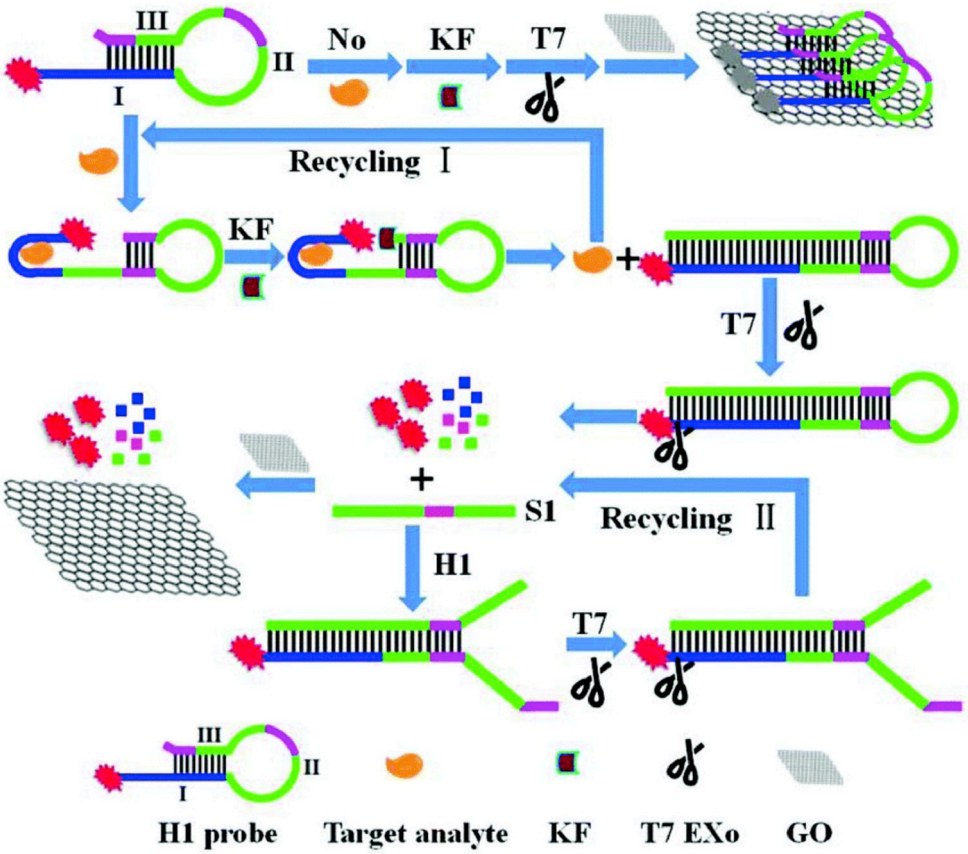

Fig. 6. Schematic diagram of single oligonucleotide-mediated isothermal secondary amplification (SOIQA). Reprinted with permission from Ref. [69].

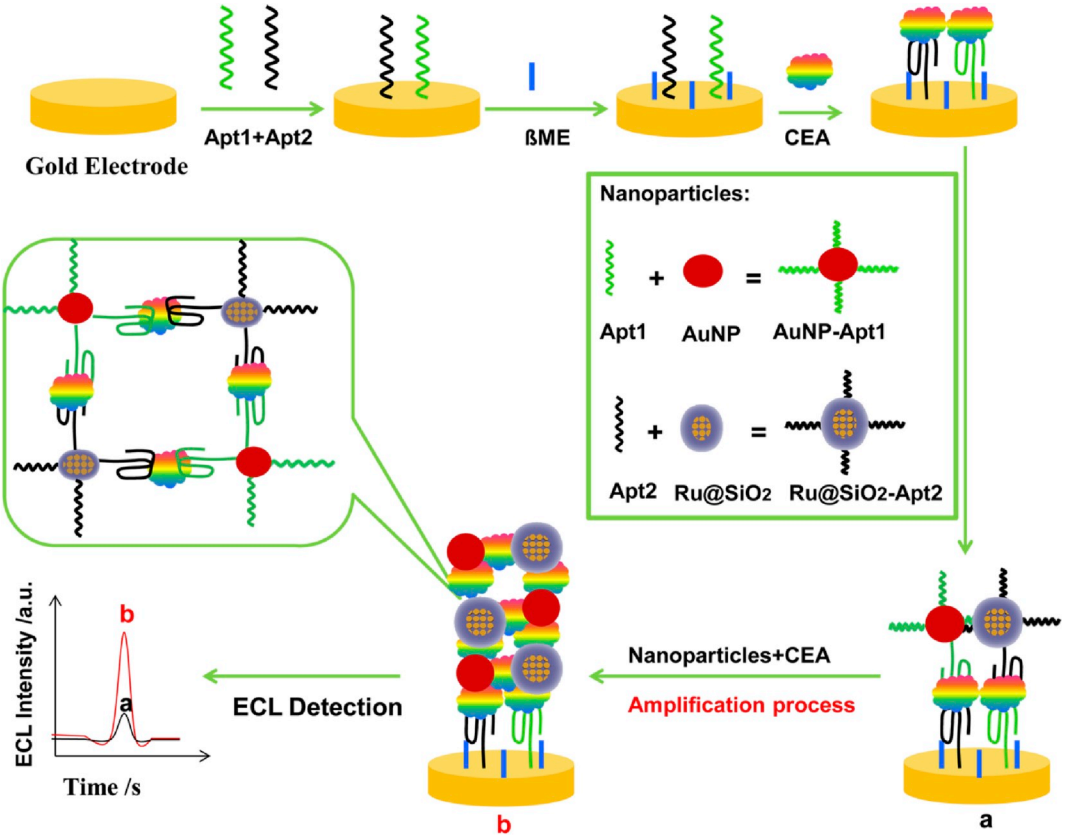

Fig. 7. Schematic diagram of CEA detection in human serum based on localized surface plasmon resonance (LSPR) enhanced electrochemiluminescence (ECL) of Ru $(bpy)_3^{2+}$. Reprinted with permission from Ref. [73]. Copyright 2015 American Chemical Society.

Table 2
Electrochemical aptamer biosensors for CEA determination.

Method	Assay strategy	nanomaterial	Sensor detection buffer	Recognition motif	Linear range	LOD	Ref
EIS	Glucose oxidase (GOx)-initiated cascade catalysis amplification	Pt@CuMOFs-hGq-GOx	PBS	ssDNA	0.05 pg mL $^{-1}$ to 20 ng mL^{-1}	0.023 pg mL $^{-1}$	[133]
	MnTMPyP-dsDNA assisted by HCR	PtPd nanowires	PBS	dsDNA	0.1 pg mL^{-1} to 0.5 ng mL^{-1} and 1 ng mL^{-1} to 40 ng mL^{-1}.	0.030 pg mL^{-1}	[134]
Amperometric	Sandwich complexes	AuNPs	PBS	ssDNA	1–200 ng mL^{-1}	0.5 ng mL^{-1}	[135]
	Aptamer-initiated on-particle template-independent enzymatic polymerization (aptamer-OTEP)	AuNP	PBS	ssDNA	50 fM–500 pM	5 fM	[136]
Voltammetric	A dual-target electrochemical	Au	PBS	dsDNA	0.01 pM to 100 nM	3.33 fM	[36]
	Dual-analyte detection system	AuNPs	PBS	integrated signal probe (ISP)	1 ng mL^{-1} to 1 μg mL^{-1}	0.517 ng mL^{-1}	[137]
	A nanocomposite of multi-walled carbon nanotubes, hemin and graphene nanosheets (HGNs-MWCNTs)	HGNs-MWCNTs	PBS	ssDNA	1 × 10^{-15} to 1 × 10^{-8} g mL^{-1}	0.82 fg m L^{-1}	[138]
	Lead ion (Pb^{2+})-dependent DNAzyme-assisted signal amplification	GQDs-IL-NF	PBS	hairpin DNA	0.5 to 500 fg mL^{-1}	0.34 fg mL^{-1}	[139]
	A shell-encoded Au NPs	Au@Cu_2O and Au@Ag core-shell NPs	PBS	dsDNA	5 pg mL^{-1} to 10 ng mL^{-1}	1.8 pg mL^{-1}	[140]
	Enzymatically catalytic signal tracing	pAu NS	Tris-HCl	ssDNA	1.0 pg mL $^{-1}$ to 100 ng mL^{-1}	0.45 pg mL^{-1}	[141]
	Ag NCs-HRP signal probe	Ag NCs	PB	ssDNA	1 pg to 10 ng mL^{-1}	0.5 pg mL^{-1}	[142]
	A ternary nanocomposite of AuNPs-HGNs	AuNPs-HGNs	PBS	ssDNA	0.0001–10 ng mL^{-1}	40 fg mL^{-1}	[143]
Potentiometric	Field-effect transistor (FET) biosensors	C-PPy MNTs	PBS	ssDNA	–	1 fg mL^{-1}	[144]
PEC	Enzyme-free cascaded quadratic amplification	CdS/TiO_2	TBE	dsDNA	8.0 fg mL^{-1} to 50.0 pg mL^{-1}	–	[145]
	RET between P–TiO_2 NA and CNTs-Au nanocomposites	CNTs-Au and P–TiO_2 NA	Tris-HCl	dsDNA	0.001–2.5 ng mL^{-1}	0.39 pg mL^{-1}	[146]
	RET between CdTe QDs and RGO-AuNPs	RGO-AuNPs and CdTe QDs	Tris-HCl	dsDNA	0.001–2.0 ng mL^{-1}	0.47 pg mL^{-1}	[147]
	$NaYF_4$:Yb, Er UCN coupling with target-triggered HCR	$NaYF_4$:Yb, Er UCN and Ag_2S	HEPES	ssDNA	0.005–5.0 ng mL^{-1}	1.9 pg mL^{-1}	[148]
	NaYF4:Yb,Tm@TiO2 upconversion microrods with RCA	$NaYF_4$: Yb , Tm @ TiO_2	PBS	ssDNA	10 pg mL^{-1} to 40 ng mL^{-1}	3.6 pg mL^{-1}	[149]
	Dual signal readout	$NaYF_4$: Yb , Er UCNPs @ CdTe and AuNPs	PBS	dsDNA	10.0 pg mL^{-1} to 5.0 ng mL^{-1}	4.8 pg mL^{-1}	[150]
	ZnO flower-rods (ZnO FRs) modified with g-C_3N_4-AuNP nanohybrids	g-C_3N_4-AuNPs and CuS NCs	Tris-HCl	dsDNA	0.01–2.5 ng mL^{-1}	1.9 pg mL^{-1}	[27]

background noise, and better signal to noise ratio [127,130]. However, the main problem limiting the development of electrochemical sensor is the detection limit which is much higher than the polymerase chain reaction (PCR) or fluorescence measurement [131]. In addition, there are some issues for electrochemical methods that deserve researchers' attention, such as reproducibility of preparations, difficulty in regenerating sensing surfaces, and often indirect sensing systems [132].

This chapter introduces electrochemical aptamer biosensors for CEA, such as electrochemical impedance spectroscopy (EIS), amperometric, voltammetric, potentiometric, and photoelectrochemical (PEC) biosensor. Table 2 summarizes the published researchs on the development of electrochemical biosensors for CEA detection.

3.1. EIS-based CEA aptamer biosensor

EIS biosensor measures the change in the electrical impedance spectrum produced by the interaction between the analyte and the receptor. The region for receptor fixation is mainly on the surface of the electrode or in the gap between the electrodes. And it can measure changes in electrical properties caused by interactions between aptamers and analytes [151,152]. EIS is not only an effective method for characterizing functionalized substrates of biomolecules, but also a sensitive technique for monitoring aptamer-ligand binding that occurs on the surface of electrodes. An important advantage of EIS biosensors is that the stimulus sinusoidal voltage is small and does not damage or interfere with most biorecognition layers [153]. Owing to its large amount of information, nondestructive, simple, and unmarked, EIS biosensor platform has received considerable attention and wide application [49,154,155]. Zhou et al. proposed a sensitive electrochemical impedimetric aptamer biosensor for CEA detection based on glucose oxidase (GOx)-driven cascade catalyzed amplification, using Pt nanoparticles, aptamers, hemin, and GOx constitutes a functionalized Cu-based metal-organic (Pt @ CuMOFs-hGq-GOx) (Fig. 8). Pt @ CuMOFs and hemin/G-quadruplex (hGq) synergistically catalyzed the decomposition of H_2O_2 produced by the cascade reaction. It was accompanied by oxidizing the 3,3-diaminobenzidine (DAB) and forming a non-conductive insoluble precipitate (IP). It resulted in a remarkable increasement in the electrochemical impedance signal. The LOD of this biosensor was 0.023 pg mL^{-1} with the range of 0.05 pg mL^{-1} to 20 ng mL^{-1} [133]. Unfortunately, this technology is still not fully understood. By using EIS as a means of supporting voltamperance evidence for electrochemical work, rather than the primary technique [156].

3.2. Amperometric -based CEA aptamer biosensor

The amperometric biosensor gauges the magnitude of current generated by a constant reduction or oxidation potential applied to the working electrode [157]. Therefore, amperometric biosensor is well suited for detecting electroactive substances involved in chemical or biometric processes [158]. Due to the inherent simplicity of the transducer, it is suitable for low-cost portable devices that often used for disease diagnosis and environmental monitoring [128].

AuNPs have excellent electrical conductivity, high surface area, and good catalytic properties, so they are ideal materials for preparing electrochemical biosensors [159,160]. Shu et al. proposed a new electrochemical aptamer biosensor based on signal amplification of AuNPs. They used two different aptamers for CEA identification. Compared with single aptamer, two different CEA aptamers produced better recognition accuracy, resulting in a lower background signal and good selectivity [135]. In another study, Wang et al. constructed an electrochemical detection system based on aptamer-initiated on-particle template-independent enzymatic polymerization (aptamer-OTEP) (Fig. 9). The aptamer 1 connected to a gold electrode was used as a capture probe, and aptamer 2 modified on the surface of gold nanoparticles was used as a nanoprobe. The capture probe and the nanoprobe formed a sandwich structure with CEA, causing the nanoprobe being attached to the electrode surface. Thereafter, high-efficiency amplification by terminal deoxynucleotidyl transferase (TdT) and avidin modified horseradish peroxidase (Av-HRP) dramatically increased the electrochemical signal [136].

3.3. Voltammetric-based CEA aptamer biosensor

The use of voltammetric sensors is of particular interest in electrochemical sensors. They use several different techniques including cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), etc. to detect low concentrations of tumor markers with high sensitivity, high specificity, and good suitability [161,162]. The voltammetric biosensor can sense low correlation noise, which can provide credible and repeatable data for quantification of analytes. This data in higher sensitivity and specificity of the voltammetric biosensor. In addition, voltammetric sensor can detect multiple analytes with different peak potentials in a single electrochemical experiment (or scan), thus providing the possibility to detect multiple substances simultaneously [36,137,140].

Mazloum-Ardakani et al. developed an aptamer biosensor based on multi-walled carbon nanotubes, hemin and graphene nanosheets (HGNs-MWCNTs) for sensitive detection of CEA. The nanocomposite could provide a comprehensive set of the main excellent properties of three nanomaterials. Due to its well-defined redox properties, Hemin was used as an in situ probe, providing a good interface between the aptamers and the surface electrode. And multi-walled carbon nanotubes in nanocomposites improved electrical conductivity and accelerated electron transfer between hemin and glassy carbon electrodes. When CEA was captured at the interface by CEA binding aptamers (CBA), the signal of hemin was further reduced because of the thickening of electron transfer. The concentration of CEA detected by the electrochemical aptamer biosensor with a wide linear range of 1.0×10^{-15} g mL^{-1} to 1.0×10^{-8} g mL^{-1}, and the detection limit was 0.82 fg mL^{-1}. This low-cost, high sensitivity and selectivity biosensor could be a hopeful means for CEA detection in clinical applications [138].

In another study, Huang et al. proposed an electrochemical aptamer biosensor using lead ion (Pb^{2+})-dependent DNAzyme-assisted signal amplification and graphene quantum dot-ionic liquid-nafion (GQDs-IL-NF) composite film for CEA electrochemical measurement (Fig. 10). This was the first time that GQDs-IL-NF composite film had been prepared on an electrode. When CEA presented, hairpin DNA recognized CEA and triggered DNAzyme-assisted signal amplification reaction to produce a large amount of single-stranded DNA which could adsorbed on the GQDs-IL-NF modified GCE by a π-π stacking interaction. Thus, The methylene blue-abeled substrate DNA (MB-substrate) is immobilized on the electrode and generated a large initial electrochemical signal [139].

Zhao et al. prepared shell-encoded AuNPs containing Au@Cu_2O and Au@Ag core-shell NP by depositing different amounts of Cu and Ag precursors. The AuNPs encoded by the Cu_2O shell and the Ag shell exhibited two independent DPV peaks at −0.08 V and 0.26 V, respectively. The shell-encoded AuNP exhibited an amplified peak current with an adjustable shell thickness. Electrochemical aptamer sensors using shell-encoded AuNPs could double screen CEA and alpha-fetoprotein (AFP) [140].

3.4. Potentiometric based CEA aptamer biosensor

The potentiometric biosensor measures the potential difference between the working electrode and the reference electrode caused by a change in the concentration of charged species. In this measurement, the potential difference is determined by the voltammeter when no significant current flows [163,164]. Field effect transistors (FET) are a special type of potentiometric biosensor [128]. When zero or negligible

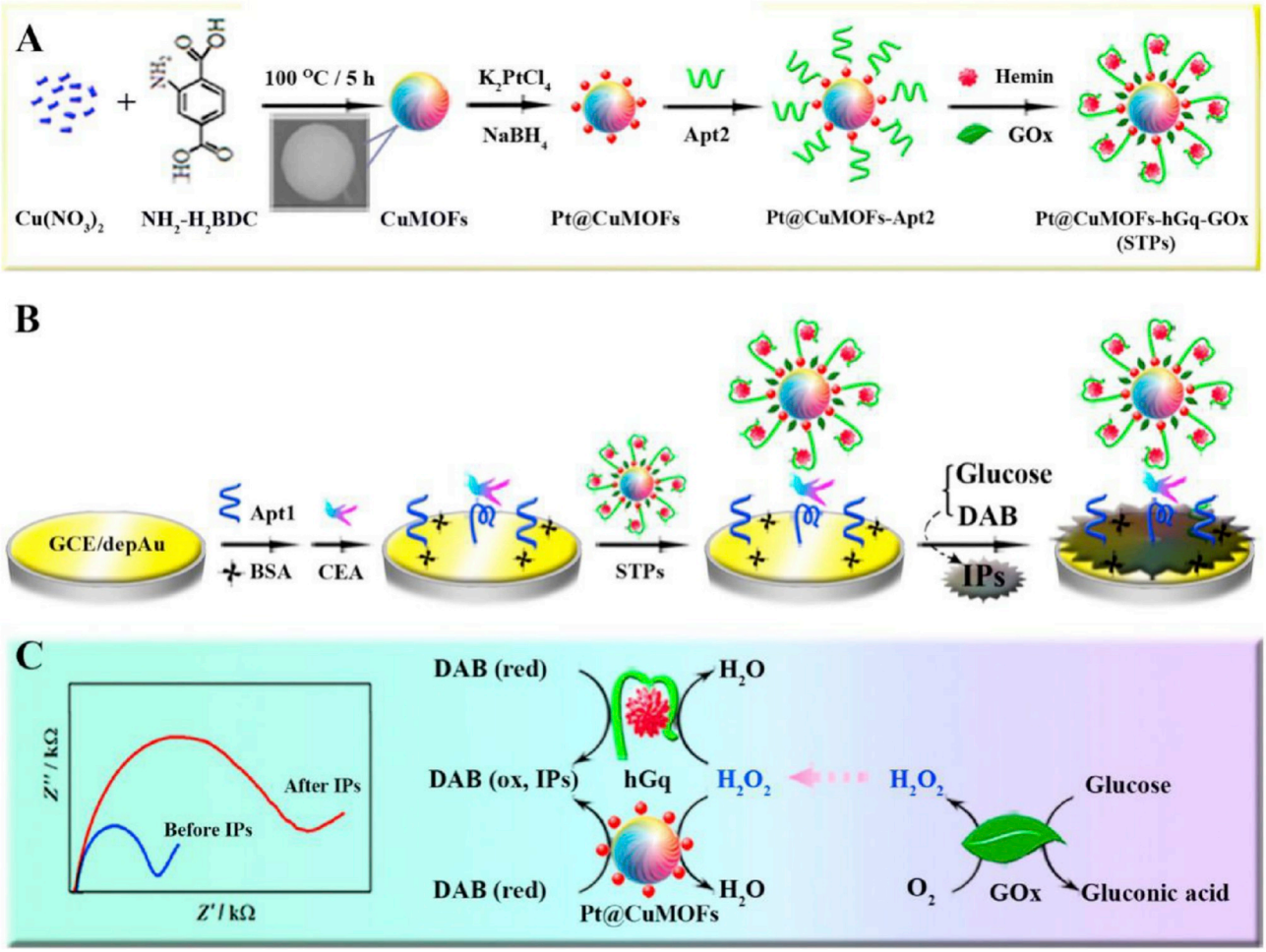

Fig. 8. Schematic diagram of EIS detection based on Glucose oxidase (GOx)-initiated cascade catalysis amplification: (A) Preparation process of Pt@CuMOFs-hGq-GOx, (B) Schematic illustration of fabrication of the impedimetric aptasensor, and (C) Cascade catalysis amplification to form nonconductive insoluble precipitates (IPs). Reprinted with permission from Ref. [133].

current flows through the electrode, the potential difference accumulates during the identification process in the electrochemical cell [165]. The combination of charged targets induces changes in the intrinsic carrier concentration of the FET channel, which allows the FET to be biometrically identified, with no labeling, high sensitivity, high selectivity, and repetitive properties [166–168]. Park et al. used aptamer-functionalized polydimensional conductive-polymer (3-carboxylate polypyrrole) nanotubes (Apt-C-PPy MNT) to fabricate a FET biosensor to detect CEA (Fig. 11). A C-PPy MNT multidimensional system was synthesized by a solution-based temperature control technique and

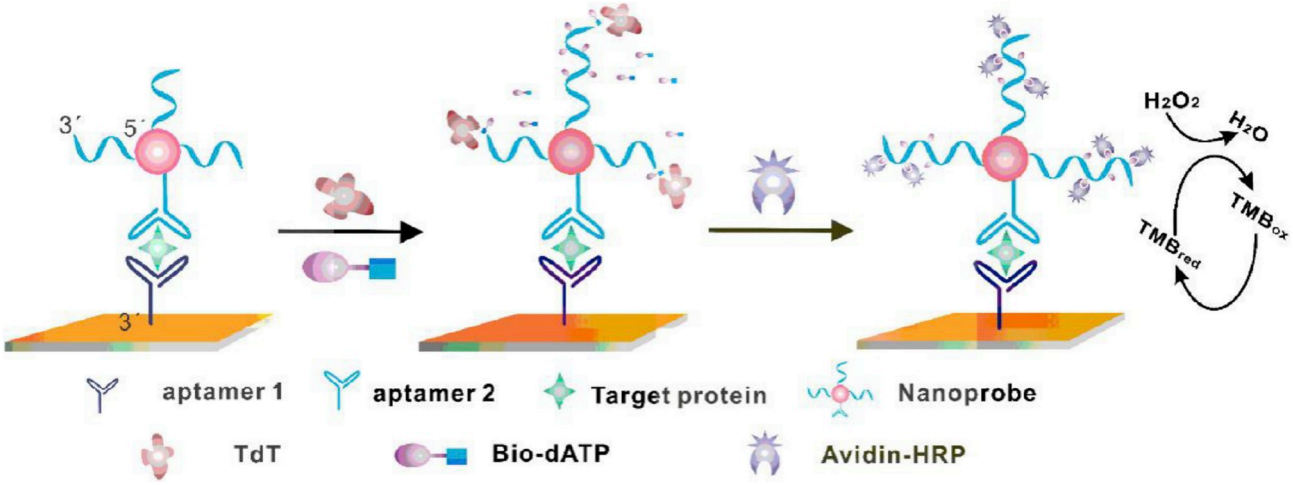

Fig. 9. Schematic diagram of an aptamer-initiated on-particle template-independent enzymatic polymerization (aptamer-OTEP) amperometric biosensor. Reprinted with permission from Ref. [136].

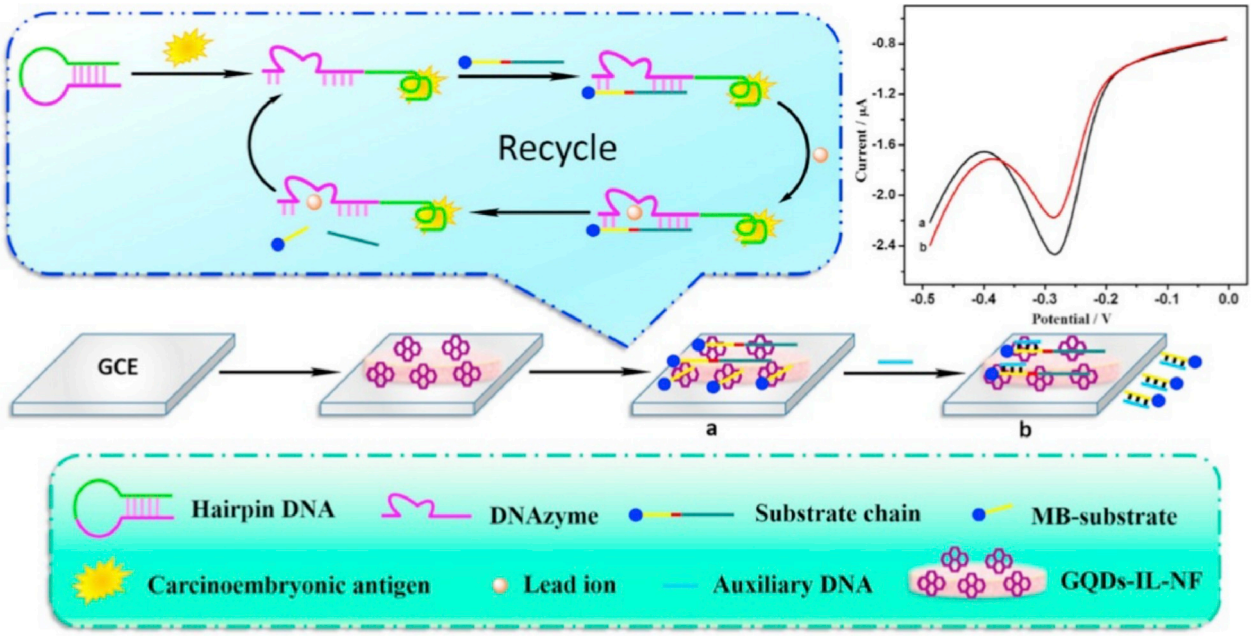

Fig. 10. Schematic diagram of CEA detection based on lead ion (Pb2 +)-dependent DNAzyme assisted signal amplification strategy. Reprinted with permission from Ref. [139].

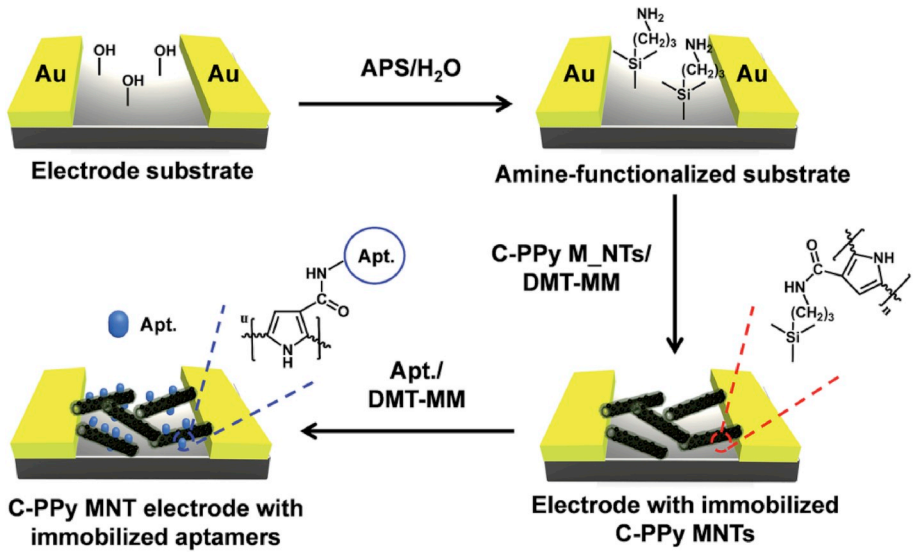

Fig. 11. Schematic diagram of a field effect transistor (FET) biosensor based aptamer-functionalized multidimensional conducting-polymer (3-carboxylate polypyrrole) nanotubes (Apt–C-PPy MNTs). Reprinted with permission from Ref. [144].

fixed to the surface of the electrode. It was then bound to the amine modified CEA aptamer via an amide covalent bond. The FET biosensor based on C-PPy MNT exhibited a fast response (< -1s) for CEA detection, which had a good electrical conductivity. This was the first demonstration of CEA detection using a multi-dimensional CPNT-based FET biosensor with a detection limit of approximately 1 fg mL^{-1} [144].

3.5. Photoelectrochemical based CEA aptamer biosensor

Photoelectrochemical (PEC) biosensors are highly efficient sensing technologies coupled with electrochemistry and photochemistry which have attracted more and more attention. The detection process of PEC biosensor is the opposite of ECL biosensor: light serves as the excitation source and photocurrent serves as the detection signal. It typically rely on the introduction of photoinduced electrons/holes into the photoactive material by photoexcitation to transfer it to the electrode interface, resulting in a change in the output photocurrent signal [169,170]. PEC biosensors, combining the advantages of optical and electrochemical methods, have a low background signal, which is more sensitive than traditional electrochemical methods and does not require the complicated and expensive instrument cost of optical biosensors [171–173].

Ge et al. developed a PEC biosensor with an enzyme-free cascaded quadratic signal amplification method using catalytic hairpin assembly (CHA) and hybridization chain reaction (HCR) for CEA detection. CEA recognized the CEA-aptamer @ sstDNA (single-stranded trigger) complex, which caused sstDNA released from the complex, thereby triggering the upstream CHA recycling loop. The dsDNA complex produced by CHA further induced downstream HCR amplification, resulting in forming many hemin/G-quadruplex DNases. This would stimulate the

insoluble/insulating products formed by the biocatalytic precipitation of 4-chloro-1-naphthol (4-CN), leading to a significant reduction in photocurrent signals [145].

In another study, Deng et al. proposed a PEC biosensor based on resonance energy transfer (RET) between pinnate titanium dioxide nanorods array (P–TiO_2 NA) and carbon nanotubes-gold nanoparticles (CNTs-Au) nanocomposites for highly sensitive detection of CEA. As the quenching efficiency of excitons generated in P–TiO_2 NA decreases, the energy transfer efficiency decreased resulting in an increase in PEC response. The PEC aptamer sensor had a linear range of 0.001–2.5 ng mL^{-1} with a detection limit of 0.39 pg mL^{-1} and was satisfactory for clinical sample testing [146].

Qiu et al. constructed three sensitive filters based on near-infrared (NIR) light-driven PEC aptamer biosensors for CEA. One of the biosensors amplified the signal by coupling with the HCR under the illumination of near-infrared light and the formation of Ag_2S nanoparticles. The PEC aptamer platform had high sensitivity to the determination of CEA in the dynamic linear range of 0.005–5.0 ng mL^{-1} with a detection limit of 1.9 pg mL^{-1} [148]. Another biosensor was based on core–shell $NaYF_4$: Yb, Tm@TiO_2 upconversion micro rods by coupling with target-triggered rolling circle amplification (RCA). The working range was 10 pg mL^{-1} to 40 ng mL^{-1} allowed for the detection of CEA as low as 3.6 pg mL^{-1} [149].

4. Other CEA testing methods

In addition to aptamer-based detection methods, other detection methods are also used in the detection of CEA, for instance immuno-based optical and electrochemical biosensor [174,175], biochip [176,177], radioimmunoassay (RIA) and immunoradiometric assay (IRMA) [24], portable biosensor [178], mass change-based piezoelectric [179] and flexural plate-wave (FPW) biosensor [180] etc..

To date, scientists are still improving the ELISA method to achieve a high sensitivity and better stability. Zhou et al. developed a sandwich format ELISA based on gold nanoparticle layer (GNPL), which used IgG as a single protein solution and CEA in human plasma as a measurement system. The GNPL-based sandwich ELISA amplified the signal and reduced the LOD compared to the ELISA of the modified plate and the commercial ELISA kit. The results indicated that GNPL modified plates could be used in sandwich format ELISA in complex media [181].

The development of imaging equipment allowed high sensitivity and selective quantitative analysis to be applied at the single molecule level [182,183] Ahn et al. combined a total internal reflection scattering microscope based on transmission grating (TG) with a plasma nano-immunosensor to improve the detection sensitivity of CEA. Scattering signals of silver nanoparticles (SNP) nanoprobes bound to CEA in EFL were collected and spectrally separated using a TG beam splitter. The combination of the two techniques minimized the interference of spectroscopic and background noise. The LOD was as low as 19.75 zM and the linear dynamic range was 19.75 zM to 39.50 nM. Especially, this strategy could be used to directly test multiple protein biomarkers at the level of a single molecule in a human biological sample by simply altering the antibody of the target protein [183].

Label-free atomic spectrometric bioassays has attracted extensive research interest due to its low cost, simple design and ease of operation. Chen et al. proposed a novel chemical vapor generation-atomic fluorescence spectrometry (CVG-AFS)/inductively coupled plasma-mass spectrometry (ICP-MS) label-free sensing method for the detection of nucleic acids and proteins (CEA). This assay retained the advantages of label-free atomic spectroscopy bioassay and combineed with selective cation exchange reaction and simple filtration separation to improve the detection performance. The LOD was 0.2 ng mL^{-1} for CEA detection with a linear dynamic range of 0.5–20 ng mL^{-1} [184].

5. Conclusion

As one of the common tumor markers, CEA has important clinical value in the differential diagnosis, disease monitoring and therapeutic evaluation of malignant tumors. In the past few decades, biosensor technology has attracted the attention of scientists due to its simple, fast, low-cost detection, high sensitivity and good selectivity. The aptamer is relatively fast and inexpensive to produce and can be chemically synthesized. It is extremely accurate and repeatable, with flexible marking and good stability. Also, aptamer targets are less restrictive than antibodies, so they have a wide range of applications in biosensors and medicine. It becomes a suitable substitute for antibodies.

This article outlines the use of aptamers in CEA biosensors. It is introduced from two aspects of aptamer-based optical and electrochemical biosensors. We analyzed the characteristics of each sensor and introduced their advantages and disadvantages. Some biosensors combined excellent nanomaterials such as graphene, metal nanoparticles, quantum dots, etc., which are widely used in optical and electrochemical biosensors. Nanomaterials have a large surface-volume ratio which can achieve efficient target interaction. In addition, the development of nanocomposites can combine the advantages of multiple nanomaterials to develop new assays with ultra-sensitivity and multi-parameter functionality. After combining nanomaterials, biosensor performance has been greatly optimized and improved, which can improve the sensitivity and specificity of detection. Due to the existence of multiple tumor markers in cancer patients, some researchers have been working on the development of biosensors that can detect multiple tumor markers at the same time. Although some efforts have been made to design biosensors for detecting multiple biomarkers simultaneously, functional verification is still limited. Complex biological sample environments can interfere with the results of the analysis and there may be false positive signals. Some of the sensors that have been developed still require dilution of the serum for sensory analysis. For mass-based piezoelectric and magnetoelectric biosensors, the related article has not yet been proposed based on the aptamer detection scheme. Furthermore, current CEA aptamer biosensors lack the research for real-time quantitative portable biosensors. Although conventional biosensors have been miniaturized and cost-effective, they have not yet achieved in vitro detection of easy-to-use, home-use, and most biosensors lack relevant research for complex environmental detection. To date, commercial CEA biosensors are still in their infancy. These questions still need to be explored by researchers.

Declaration of competing interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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References

[1] D. Di Gioia, I. Blankenburg, D. Nagel, V. Heinemann, P. Stieber, Tumor markers in the early detection of tumor recurrence in breast cancer patients: CA 125, CYFRA 21-1, HER2 shed antigen, LDH and CRP in combination with CEA and CA 15-3, Clin. Chim. Acta 461 (2016) 1–7.

[2] M.J. Duffy, R. Lamerz, C. Haglund, A. Nicolini, M. Kalousova, L. Holubec, C. Sturgeon, Tumor markers in colorectal cancer, gastric cancer and gastrointestinal stromal cancers: European group on tumor markers 2014 guidelines update, Int. J. Cancer 134 (2014) 2513–2522.

[3] H. Shimada, T. Noie, M. Ohashi, K. Oba, Y. Takahashi, Clinical significance of serum tumor markers for gastric cancer: a systematic review of literature by the Task Force of the Japanese Gastric Cancer Association, Gastric Cancer 17 (2014) 26–33.

[4] Y. Lai, L. Wang, Y. Liu, G. Yang, C. Tang, Y. Deng, S. Li, Immunosensors based on nanomaterials for detection of tumor markers, J. Biomed. Nanotechnol. 14 (2018) 44–65.
[5] G. Lech, R. Slotwinski, M. Slodkowski, I.W. Krasnodebski, Colorectal cancer tumour markers and biomarkers: recent therapeutic advances, World J. Gastroenterol. 22 (2016) 1745–1755.
[6] E. Wieczorek, E. Reszka, mRNA, microRNA and lncRNA as novel bladder tumor markers, Clin. Chim. Acta 477 (2018) 141–153.
[7] X. Han, J. Wang, Y. Sun, Circulating tumor DNA as biomarkers for cancer detection, Genom. Proteom. Bioinform. 15 (2017) 59–72.
[8] B.K. Banin Hirata, J.M. Oda, R. Losi Guembarovski, C.B. Ariza, C.E. de Oliveira, M.A. Watanabe, Molecular markers for breast cancer: prediction on tumor behavior, Dis. Markers (2014) (2014) 513158.
[9] M.H. Tan, H.Y. Wang, C.H. Hsieh, C.N. Wen, Y.H. Wen, C.H. Chen, J.J. Lu, Cancers screening in an asymptomatic population by using multiple tumour markers, PLoS One 11 (2016).
[10] P. Gold, S.O. F, Specific carcinoembryonic antigens of the human digestive system, J. Exp. Med. 122 (1965) 467–481.
[11] C.H. Chen, M.C. Hsieh, C.C. Lai, C.Y. Yeh, J.S. Chen, P.S. Hsieh, J.M. Chiang, W.S. Tsai, R. Tang, C.R. Changchien, J.Y. Wang, Lead time of carcinoembryonic antigen elevation in the postoperative follow-up of colorectal cancer did not affect the survival rate after recurrence, Int. J. Colorectal Dis. 25 (2010) 567–571.
[12] A. Chiaravalloti, A. Fiorentini, E. Palombo, D. Rinino, A. Lacanfora, R. Danieli, C. Di Russo, D. Di Biagio, E. Squillaci, O. Schillaci, Evaluation of recurrent disease in the re-staging of colorectal cancer by (18)F-FDG PET/CT: use of CEA and CA 19-9 in patient selection, Oncol Lett 12 (2016) 4209–4213.
[13] J.A. Hensel, V. Khattar, R. Ashton, S. Ponnazhagan, Recombinant AAV-CEA tumor vaccine in combination with an immune adjuvant breaks tolerance and provides protective immunity, Mol Ther Oncolytics 12 (2019) 41–48.
[14] R.D. Blumenthal, E. Leon, H.J. Hansen, D.M. Goldenberg, Expression patterns of CEACAM5 and CEACAM6 in primary and metastatic cancers, BMC Canc. 7 (2007) 2.
[15] J. Marshall, Carcinoembryonic antigen-based vaccines, Semin. Oncol. 30 (2003) 30–36.
[16] K.M. Yang, I.J. Park, C.W. Kim, S.A. Roh, D.H. Cho, J.C. Kim, The prognostic significance and treatment modality for elevated pre- and postoperative serum CEA in colorectal cancer patients, Ann Surg Treat Res 91 (2016) 165–171.
[17] C.O. Sahlmann, K. Homayounfar, M. Niessner, J. Dyczkowski, L.C. Conradi, F. Braulke, B. Meller, T. Beissbarth, B.M. Ghadimi, J. Meller, D.M. Goldenberg, T. Liersch, Repeated adjuvant anti-CEA radioimmunotherapy after resection of colorectal liver metastases: safety, feasibility, and long-term efficacy results of a prospective phase 2 study, Cancer 123 (2017) 638–649.
[18] G. Saito, S. Sadahiro, K. Okada, A. Tanaka, T. Suzuki, A. Kamijo, Relation between carcinoembryonic antigen levels in colon cancer tissue and serum carcinoembryonic antigen levels at initial surgery and recurrence, Oncology 91 (2016) 85–89.
[19] K.G. Spindler, C. Demuth, B.S. Sorensen, J.S. Johansen, D. Nielsen, N. Pallisgaard, E. Hoegdall, P. Pfeiffer, B. Vittrup Jensen, Total cell-free DNA, carcinoembryonic antigen, and C-reactive protein for assessment of prognosis in patients with metastatic colorectal cancer, Tumour Biol 40 (2018) 1010428318811207.
[20] E. Tan, N. Gouvas, R.J. Nicholls, P. Ziprin, E. Xynos, P.P. Tekkis, Diagnostic precision of carcinoembryonic antigen in the detection of recurrence of colorectal cancer, Surg Oncol 18 (2009) 15–24.
[21] S. Yokoyama, A. Takeuchi, S. Yamaguchi, Y. Mitani, T. Watanabe, K. Matsuda, T. Hotta, J.E. Shively, H. Yamaue, Clinical implications of carcinoembryonic antigen distribution in serum exosomal fraction-Measurement by ELISA, PLoS One 12 (2017) e0183337.
[22] W. Yang, T. Huang, M. Zhao, F. Luo, W. Weng, Q. Wei, Z. Lin, G. Chen, High peroxidase-like activity of iron and nitrogen co-doped carbon dots and its application in immunosorbent assay, Talanta 164 (2017) 1–6.
[23] X. Gu, Z. She, T. Ma, S. Tian, H.B. Kraatz, Electrochemical detection of carcinoembryonic antigen, Biosens. Bioelectron. 102 (2018) 610–616.
[24] B.D. Nicholson, B. Shinkins, N.W. Roberts, T.J. James, S. Mallett, R. Perera, J.N. Primrose, D. Mant, Blood CEA levels for detecting recurrent colorectal cancer, Cochrane Database Syst. Rev. (2015) (2015) CD011134.
[25] L. Guo, Y. Shi, X. Liu, Z. Han, Z. Zhao, Y. Chen, W. Xie, X. Li, Enhanced fluorescence detection of proteins using ZnO nanowires integrated inside microfluidic chips, Biosens. Bioelectron. 99 (2018) 368–374.
[26] L. Xiao, A. Zhu, Q. Xu, Y. Chen, J. Xu, J. Weng, Colorimetric biosensor for detection of cancer biomarker by Au nanoparticle-decorated Bi2Se3 nanosheets, ACS Appl. Mater. Interfaces 9 (2017) 6931–6940.
[27] Z. Han, M. Luo, Q. Weng, L. Chen, J. Chen, C. Li, Y. Zhou, L. Wang, ZnO flower-rod/g-C3N4-gold nanoparticle-based photoelectrochemical aptasensor for detection of carcinoembryonic antigen, Anal. Bioanal. Chem. 410 (2018) 6529–6538.
[28] T.Y. Xing, J. Zhao, G.J. Weng, J. Zhu, J.J. Li, J.W. Zhao, Specific detection of carcinoembryonic antigen based on fluorescence quenching of hollow porous gold nanoshells with roughened surface, ACS Appl. Mater. Interfaces 9 (2017) 36632–36641.
[29] R. Li, F. Feng, Z.Z. Chen, Y.F. Bai, F.F. Guo, F.Y. Wu, G. Zhou, Sensitive detection of carcinoembryonic antigen using surface plasmon resonance biosensor with gold nanoparticles signal amplification, Talanta 140 (2015) 143–149.
[30] V. Crivianu-Gaita, M. Thompson, Aptamers, antibody scFv, and antibody Fab' fragments: an overview and comparison of three of the most versatile biosensor biorecognition elements, Biosens. Bioelectron. 85 (2016) 32–45.
[31] M. Singh, N. Verma, A. Garg, N. Redhu, Urea biosensors, Sens. Actuators B Chem. 134 (2008) 345–351.
[32] S. Dehghani, R. Nosrati, M. Yousefi, A. Nezami, F. Soltani, S.M. Taghdisi, K. Abnous, M. Alibolandi, M. Ramezani, Aptamer-based biosensors and nanosensors for the detection of vascular endothelial growth factor (VEGF): a review, Biosens. Bioelectron. 110 (2018) 23–37.
[33] N. Verma, A. Bhardwaj, Biosensor technology for pesticides–a review, Appl. Biochem. Biotechnol. 175 (2015) 3093–3119.
[34] I. Willner, M. Zayats, Electronic aptamer-based sensors, Angew Chem. Int. Ed. Engl. 46 (2007) 6408–6418.
[35] J. Ali, J. Najeeb, M. Asim Ali, M. Farhan Aslam, A. Raza, Biosensors: their fundamentals, designs, types and most recent impactful applications: a review, J. Biosens. Bioelectron. 8 (2017).
[36] C. Ma, H. Liu, L. Zhang, H. Li, M. Yan, X. Song, J. Yu, Multiplexed aptasensor for simultaneous detection of carcinoembryonic antigen and mucin-1 based on metal ion electrochemical labels and Ru(NH3)6(3+) electronic wires, Biosens. Bioelectron. 99 (2018) 8–13.
[37] M. Ahmadzadeh-Raji, E. Ghafar-Zadeh, G. Amoabediny, An optically-transparent aptamer-based detection system for colon cancer applications using gold nanoparticles electrodeposited on indium tin oxide, Sensors (2016) 16.
[38] H. Khang, K. Cho, S. Chong, J.H. Lee, All-in-one dual-aptasensor capable of rapidly quantifying carcinoembryonic antigen, Biosens. Bioelectron. 90 (2017) 46–52.
[39] T. Hermann, D.J. Pate1, Adaptive recognition by nucleic acid aptamers, Science 287 (2000) 820–825.
[40] L. Wen, L. Qiu, Y. Wu, X. Hu, X. Zhang, Aptamer-modified semiconductor quantum dots for biosensing applications, Sensors 17 (2017).
[41] P. Kalra, A. Dhiman, W.C. Cho, J.G. Bruno, T.K. Sharma, Simple methods and rational design for enhancing aptamer sensitivity and specificity, Front Mol Biosci 5 (2018) 41.
[42] J. Bala, S. Chinnapaiyan, R.K. Dutta, H. Unwalla, Aptamers in HIV research diagnosis and therapy, RNA Biol. 15 (2018) 327–337.
[43] S. Catuogno, C.L. Esposito, V. de Franciscis, Aptamer-mediated targeted delivery of therapeutics: an update, Pharmaceuticals (2016) 9.
[44] M. Yüce, N. Ullah, H. Budak, Trends in aptamer selection methods and applications, Analyst 140 (2015) 5379–5399.
[45] C. Perez-Gonzalez, D.A. Lafontaine, J.C. Penedo, Fluorescence-based strategies to investigate the structure and dynamics of aptamer-ligand complexes, Front Chem 4 (2016) 33.
[46] N. Duan, S. Wu, S. Dai, T. Miao, J. Chen, Z. Wang, Simultaneous detection of pathogenic bacteria using an aptamer based biosensor and dual fluorescence resonance energy transfer from quantum dots to carbon nanoparticles, Microchimica Acta 182 (2014) 917–923.
[47] S.C. Gopinath, T. Lakshmipriya, Y. Chen, W.M. Phang, U. Hashim, Aptamer-based 'point-of-care testing, Biotechnol. Adv. 34 (2016) 198–208.
[48] S.M. Nimjee, R.R. White, R.C. Becker, B.A. Sullenger, Aptamers as therapeutics, Nat. Rev. Drug Discov. 57 (2017) 61–79.
[49] V.-T. Nguyen, Y. Kwon, M. Gu, Aptamer-based Environmental Biosensors for Small Molecule Contaminants, (2017).
[50] L. Gao, Q. Li, R. Li, Z. Deng, B. Brady, N. Xia, G. Chen, Y. Zhou, H. Xia, K. Chen, H. Shi, Protein determination using graphene oxide-aptamer modified gold nanoparticles in combination with Tween 80, Anal. Chim. Acta 941 (2016) 80–86.
[51] L. Lan, Y. Yao, J. Ping, Y. Ying, Recent progress in nanomaterial-based optical aptamer assay for the detection of food chemical contaminants, ACS Appl. Mater. Interfaces 9 (2017) 23287–23301.
[52] P. Damborsky, J. Svitel, J. Katrlik, Optical biosensors, Essays Biochem 60 (2016) 91–100.
[53] L. Lan, Y. Yao, J. Ping, Y. Ying, Recent advances in nanomaterial-based biosensors for antibiotics detection, Biosens. Bioelectron. 91 (2017) 504–514.
[54] F. Long, A. Zhu, H. Shi, Recent advances in optical biosensors for environmental monitoring and early warning, Sensors 13 (2013) 13928–13948.
[55] C. Luo, W. Wen, F. Lin, X. Zhang, H. Gu, S. Wang, Simplified aptamer-based colorimetric method using unmodified gold nanoparticles for the detection of carcinoma embryonic antigen, RSC Adv. 5 (2015) 10994–10999.
[56] K. Liang, S. Zhai, Z. Zhang, X. Fu, J. Shao, Z. Lin, B. Qiu, G.-n. Chen, Ultrasensitive colorimetric carcinoembryonic antigen biosensor based on hyperbranched rolling circle amplification, The Analyst 139 (2014) 4330–4334.
[57] N. Shahbazi, S. Hosseinkhani, B. Ranjbar, A facile and rapid aptasensor based on split peroxidase DNAzyme for visual detection of carcinoembryonic antigen in saliva, Sens. Actuators B Chem. 253 (2017) 794–803.
[58] C. Guo, F. Su, Y. Song, B. Hu, M. Wang, L. He, D. Peng, Z. Zhang, Aptamer-templated silver nanoclusters embedded in zirconium metal-organic framework for bifunctional electrochemical and SPR aptasensors toward carcinoembryonic antigen, ACS Appl. Mater. Interfaces 9 (2017) 41188–41199.
[59] Z. Qiu, J. Shu, D. Tang, Bioresponsive release system for visual fluorescence detection of carcinoembryonic antigen from mesoporous silica nanocontainers mediated optical color on quantum dot-enzyme-impregnated paper, Anal. Chem. 89 (2017) 5152–5160.
[60] X. Yang, Y. Zhuo, S. Zhu, Y. Luo, Y. Feng, Y. Xu, Selectively assaying CEA based on a creative strategy of gold nanoparticles enhancing silver nanoclusters' fluorescence, Biosens. Bioelectron. 64 (2015) 345–351.
[61] H. Miao, L. Wang, Y. Zhuo, Z. Zhou, X. Yang, Label-free fluorimetric detection of CEA using carbon dots derived from tomato juice, Biosens. Bioelectron. 86 (2016) 83–89.
[62] B. Bao, P. Su, J. Zhu, J. Chen, Y. Xu, B. Gu, Y. Liu, L. Wang, Rapid aptasensor capable of simply detect tumor markers based on conjugated polyelectrolytes, Talanta 190 (2018) 204–209.
[63] J. Xu, W. Chen, M. Shi, Y. Huang, L. Fang, S. Zhao, L. Yao, H. Liang, An aptamer-based four-color fluorometic method for simultaneous determination and imaging

of alpha-fetoprotein, vascular endothelial growth factor-165, carcinoembryonic antigen and human epidermal growth factor receptor 2 in living cells, Mikrochim. Acta 186 (2019) 204.
[64] Y. Wang, Z. Wei, X. Luo, Q. Wan, R. Qiu, S. Wang, An ultrasensitive homogeneous aptasensor for carcinoembryonic antigen based on upconversion fluorescence resonance energy transfer, Talanta 195 (2019) 33–39.
[65] H. Li, L. Shi, D.E. Sun, P. Li, Z. Liu, Fluorescence resonance energy transfer biosensor between upconverting nanoparticles and palladium nanoparticles for ultrasensitive CEA detection, Biosens. Bioelectron. 86 (2016) 791–798.
[66] Z.M. Zhou, J. Zhou, J. Chen, R.N. Yu, M.Z. Zhang, J.T. Song, Y.D. Zhao, Carcinoembryonic antigen detection based on fluorescence resonance energy transfer between quantum dots and graphene oxide, Biosens. Bioelectron. 59 (2014) 397–403.
[67] Y. Sun, J. Fan, L. Cui, W. Ke, F. Zheng, Y. Zhao, Fluorometric nanoprobes for simultaneous aptamer-based detection of carcinoembryonic antigen and prostate specific antigen, Mikrochim. Acta 186 (2019) 152.
[68] Z. Lin, G. Zhang, W. Yang, B. Qiu, G. Chen, CEA fluorescence biosensor based on the FRET between polymer dots and Au nanoparticles, Chem Commun (Camb) 48 (2012) 9918–9920.
[69] J. Xu, M. Shi, H. Huang, K. Hu, W. Chen, Y. Huang, S. Zhao, A fluorescent aptasensor based on single oligonucleotide-mediated isothermal quadratic amplification and graphene oxide fluorescence quenching for ultrasensitive protein detection, Analyst 143 (2018) 3918–3925.
[70] W. Yang, X. Zhou, J. Zhao, W. Xu, A cascade amplification strategy of catalytic hairpin assembly and hybridization chain reaction for the sensitive fluorescent assay of the model protein carcinoembryonic antigen, Microchimica Acta 185 (2018).
[71] M.Q. He, K. Wang, W.J. Wang, Y.L. Yu, J.H. Wang, Smart DNA machine for carcinoembryonic antigen detection by exonuclease III-assisted target recycling and DNA walker cascade amplification, Anal. Chem. 89 (2017) 9292–9298.
[72] Z.M. Zhou, Z. Feng, J. Zhou, B.Y. Fang, X.X. Qi, Z.Y. Ma, B. Liu, Y.D. Zhao, X.B. Hu, Capillary electrophoresis-chemiluminescence detection for carcino-embryonic antigen based on aptamer/graphene oxide structure, Biosens. Bioelectron. 64 (2015) 493–498.
[73] D. Wang, Y. Li, Z. Lin, B. Qiu, L. Guo, Surface-enhanced electrochemiluminescence of Ru@SiO2 for ultrasensitive detection of carcinoembryonic antigen, Anal. Chem. 87 (2015) 5966–5972.
[74] Y.L. Wang, J.T. Cao, Y.H. Chen, Y.M. Liu, A label-free electrochemiluminescence aptasensor for carcinoembryonic antigen detection based on electrodeposited ZnS–CdS on MoS_2 decorated electrode, Analytical Methods 8 (2016) 5242–5247.
[75] G.F. Shi, J.T. Cao, J.J. Zhang, K.J. Huang, Y.M. Liu, Y.H. Chen, S.W. Ren, Aptasensor based on tripetalous cadmium sulfide-graphene electrochemiluminescence for the detection of carcinoembryonic antigen, Analyst 139 (2014) 5827–5834.
[76] X. Zhang, N. Bao, X. Luo, S.N. Ding, Patchy gold coated Fe_3O_4 nanospheres with enhanced catalytic activity applied for paper-based bipolar electrode-electrochemiluminescence aptasensors, Biosens. Bioelectron. 114 (2018) 44–51.
[77] F. Qu, T. Li, M. Yang, Colorimetric platform for visual detection of cancer biomarker based on intrinsic peroxidase activity of graphene oxide, Biosens. Bioelectron. 26 (2011) 3927–3931.
[78] H. Aldewachi, T. Chalati, M.N. Woodroofe, N. Bricklebank, B. Sharrack, P. Gardiner, Gold nanoparticle-based colorimetric biosensors, Nanoscale 10 (2017) 18–33.
[79] C. Feng, S. Dai, L. Wang, Optical aptasensors for quantitative detection of small biomolecules: a review, Biosens. Bioelectron. 59 (2014) 64–74.
[80] C. Yang, Y. Wang, J.L. Marty, X. Yang, Aptamer-based colorimetric biosensing of Ochratoxin A using unmodified gold nanoparticles indicator, Biosens. Bioelectron. 26 (2011) 2724–2727.
[81] J. Liu, Y. Lu, A colorimetric lead biosensor using DNAzyme-directed assembly of gold nanoparticles, J. Am. Chem. Soc. 125 (2003) 6642–6643.
[82] F. Sang, X. Zhang, J. Liu, S. Yin, Z. Zhang, A label-free hairpin aptamer probe for colorimetric detection of adenosine triphosphate based on the anti-aggregation of gold nanoparticles, Spectrochim. Acta A Mol. Biomol. Spectrosc. 217 (2019) 122–127.
[83] B. Liedberg, C. Nylander, I. Lunström, Surface plasmon resonance for gas detection and biosensing, Sens. Actuators 4 (1983) 299–304.
[84] C. Lertvachirapaiboon, A. Baba, S. Ekgasit, K. Shinbo, K. Kato, F. Kaneko, Transmission surface plasmon resonance techniques and their potential biosensor applications, Biosens. Bioelectron. 99 (2018) 399–415.
[85] C. Esseghaier, G.A.R.Y. Suaifan, A. Ng, M. Zourob, One-step assay for optical prostate specific antigen detection using magnetically engineered responsive thin film, J. Biomed. Nanotechnol. 10 (2014) 1123–1129.
[86] E. Wijaya, C. Lenaerts, S. Maricot, J. Hastanin, S. Habraken, J.-P. Vilcot, R. Boukherroub, S. Szunerits, Surface plasmon resonance-based biosensors: from the development of different SPR structures to novel surface functionalization strategies, Curr. Opin. Solid State Mater. Sci. 15 (2011) 208–224.
[87] H. Wang, X. Wang, J. Wang, W. Fu, C. Yao, A SPR biosensor based on signal amplification using antibody-QD conjugates for quantitative determination of multiple tumor markers, Sci. Rep. 6 (2016).
[88] Y. Wang, S. Zhang, C. Zhang, Z. Zhao, X. Zheng, L. Xue, J. Liu, X.C. Yuan, Investigation of an SPR biosensor for determining the influence of connexin 43 expression on the cytotoxicity of cisplatin, Analyst 141 (2016) 3411–3420.
[89] Q. Wang, Q. Li, X. Yang, K. Wang, S. Du, H. Zhang, Y.J.B. Nie, Bioelectronics, Graphene oxide–gold nanoparticles hybrids-based surface plasmon resonance for sensitive detection of microRNA, Biosens. Bioelectron. 77 (2016) 1001–1007.
[90] M. Mahmoudpour, J. Ezzati Nazhad Dolatabadi, M. Torbati, A. Homayouni-Rad, Nanomaterials based surface plasmon resonance signal enhancement for detection of environmental pollution, Biosens. Bioelectron. 127 (2019) 72–84.
[91] C. Liu, X. Zeng, Z. An, Y. Yang, M. Eisenbaum, X. Gu, J.M. Jornet, G.K. Dy, M.E. Reid, Q. Gan, Y. Wu, Sensitive detection of exosomal proteins via a compact surface plasmon resonance biosensor for cancer diagnosis, ACS Sens. 3 (2018) 1471–1479.
[92] H. Chen, Y. Hou, F. Qi, J. Zhang, K. Koh, Z. Shen, G. Li, Detection of vascular endothelial growth factor based on rolling circle amplification as a means of signal enhancement in surface plasmon resonance, Biosens. Bioelectron. 61 (2014) 83–87.
[93] Y. Jeong, Y.M. Kook, K. Lee, W.G. Koh, Metal enhanced fluorescence (MEF) for biosensors: general approaches and a review of recent developments, Biosens. Bioelectron. 111 (2018) 102–116.
[94] O. Tagit, N. Hildebrandt, Fluorescence sensing of circulating diagnostic biomarkers using molecular probes and nanoparticles, ACS Sens. 2 (2017) 31–45.
[95] N. Razmi, B. Baradaran, M. Hejazi, M. Hasanzadeh, J. Mosafer, A. Mokhtarzadeh, M. de la Guardia, Recent advances on aptamer-based biosensors to detection of platelet-derived growth factor, Biosens. Bioelectron. 113 (2018) 58–71.
[96] A.B. Chinen, C.M. Guan, J.R. Ferrer, S.N. Barnaby, T.J. Merkel, C.A. Mirkin, Nanoparticle probes for the detection of cancer biomarkers, cells, and tissues by fluorescence, Chem. Rev. 115 (2015) 10530–10574.
[97] Y. Han, J. Chen, Z. Li, H. Chen, H. Qiu, Recent progress and prospects of alkaline phosphatase biosensor based on fluorescence strategy, Biosens. Bioelectron. 148 (2019) 111811.
[98] M. Citartan, T.H. Tang, Recent developments of aptasensors expedient for point-of-care (POC) diagnostics, Talanta 199 (2019) 556–566.
[99] R.E. Wang, Y. Zhang, J. Cai, W. Cai, T. Gao, Aptamer-based fluorescent biosensors, Curr. Med. Chem. 18 (2011).
[100] N. Xia, F. Feng, C. Liu, R. Li, W. Xiang, H. Shi, L. Gao, The detection of mercury ion using DNA as sensors based on fluorescence resonance energy transfer, Talanta 192 (2019) 500–507.
[101] V.T. Forster, Zwischenmolekulare energiewanderung und fluoreszenz, Ann Phys-Berlin 437 (1948) 55–75.
[102] A.A. Jamali, M. Pourhassan-Moghaddam, J.E.N. Dolatabadi, Y. Omidi, Nanomaterials on the road to microRNA detection with optical and electrochemical nanobiosensors, Trac. Trends Anal. Chem. 55 (2014) 24–42.
[103] M. Fernandez-Suarez, A.Y. Ting, Fluorescent probes for super-resolution imaging in living cells, Nat. Rev. Mol. Cell Biol. 9 (2008) 929–943.
[104] K. Mao, H. Zhang, Z. Wang, H. Cao, K. Zhang, X. Li, Z. Yang, Nanomaterial-based aptamer sensors for arsenic detection, Biosens. Bioelectron. 148 (2019) 111785.
[105] H.H. Gorris, U. Resch-Genger, Perspectives and challenges of photon-upconversion nanoparticles - Part II: bioanalytical applications, Anal. Bioanal. Chem. 409 (2017) 5875–5890.
[106] X. Wang, R.R. Valiev, T.Y. Ohulchanskyy, H. Agren, C. Yang, G. Chen, Dye-sensitized lanthanide-doped upconversion nanoparticles, Chem. Soc. Rev. 46 (2017) 4150–4167.
[107] Z. Xue, Y. Zhang, W. Yu, J. Zhang, J. Wang, F. Wan, Y. Kim, Y. Liu, X. Kou, Recent advances in aflatoxin B1 detection based on nanotechnology and nanomaterials-A review, Anal. Chim. Acta 1069 (2019) 1–27.
[108] M. Wu, Q. Lai, Q. Ju, L. Li, H.D. Yu, W. Huang, Paper-based fluorogenic devices for in vitro diagnostics, Biosens. Bioelectron. 102 (2018) 256–266.
[109] Z. Zhang, S. Shikha, J. Liu, J. Zhang, Q. Mei, Y. Zhang, Upconversion nanoprobes: recent advances in sensing applications, Anal. Chem. 91 (2019) 548–568.
[110] D. Mendez-Gonzalez, E. Lopez-Cabarcos, J. Rubio-Retama, M. Laurenti, Sensors and bioassays powered by upconverting materials, Adv. Colloid Interface Sci. 249 (2017) 66–87.
[111] L. Wang, R. Wang, H. Wei, Y. Li, Selection of aptamers against pathogenic bacteria and their diagnostics application, World J. Microbiol. Biotechnol. 34 (2018) 149.
[112] C.T. Matea, T. Mocan, F. Tabaran, T. Pop, O. Mosteanu, C. Puia, C. Iancu, L. Mocan, Quantum dots in imaging, drug delivery and sensor applications, Int. J. Nanomed. 12 (2017) 5421–5431.
[113] R. Shandilya, A. Bhargava, N. Bunkar, R. Tiwari, I.Y. Goryacheva, P.K. Mishra, Nanobiosensors: point-of-care approaches for cancer diagnostics, Biosens. Bioelectron. 130 (2019) 147–165.
[114] T.H. Fang, N. Ramalingam, D. Xian-Dui, T.S. Ngin, Z. Xianting, A.T. Lai Kuan, E.Y. Peng Huat, G. Hai-Qing, Real-time PCR microfluidic devices with concurrent electrochemical detection, Biosens. Bioelectron. 24 (2009) 2131–2136.
[115] L. Liu, D. Yang, G. Liu, Signal amplification strategies for paper-based analytical devices, Biosens. Bioelectron. 136 (2019) 60–75.
[116] H. Qi, S. Yue, S. Bi, C. Ding, W. Song, Isothermal exponential amplification techniques: from basic principles to applications in electrochemical biosensors, Biosens. Bioelectron. 110 (2018) 207–217.
[117] J. Li, J. Macdonald, Advances in isothermal amplification: novel strategies inspired by biological processes, Biosens. Bioelectron. 64 (2015) 196–211.
[118] M.C. Giuffrida, G. Spoto, Integration of isothermal amplification methods in microfluidic devices: recent advances, Biosens. Bioelectron. 90 (2017) 174–186.
[119] A. Roda, M. Mirasoli, E. Michelini, M. Di Fusco, M. Zangheri, L. Cevenini, B. Roda, P. Simoni, Progress in chemical luminescence-based biosensors: a critical review, Biosens. Bioelectron. 76 (2016) 164–179.
[120] Z. Liu, F. Zhao, S. Gao, J. Shao, H. Chang, The applications of gold nanoparticle-initialed chemiluminescence in biomedical detection, Nanoscale Res Lett 11 (2016) 460.
[121] N. Li, D. Liu, H. Cui, Metal-nanoparticle-involved chemiluminescence and its applications in bioassays, Anal. Bioanal. Chem. 406 (2014) 5561–5571.
[122] J. Ping, Y. Zhou, Y. Wu, V. Papper, S. Boujday, R.S. Marks, T.W. Steele, Recent advances in aptasensors based on graphene and graphene-like nanomaterials,

Biosens. Bioelectron. 64 (2015) 373–385.
[123] L. Hu, G. Xu, Applications and trends in electrochemiluminescence, Chem. Soc. Rev. 39 (2010) 3275–3304.
[124] Y. Nasiri Khonsari, S. Sun, Recent trends in electrochemiluminescence aptasensors and their applications, Chem. Commun. 53 (2017) 9042–9054.
[125] K. Muzyka, Current trends in the development of the electrochemiluminescent immunosensors, Biosens. Bioelectron. 54 (2014) 393–407.
[126] M.R. Saidur, A.R. Aziz, W.J. Basirun, Recent advances in DNA-based electrochemical biosensors for heavy metal ion detection: a review, Biosens. Bioelectron. 90 (2017) 125–139.
[127] J.M. Moon, N. Thapliyal, K.K. Hussain, R.N. Goyal, Y.B. Shim, Conducting polymer-based electrochemical biosensors for neurotransmitters: a review, Biosens. Bioelectron. 102 (2018) 540–552.
[128] J.L. Hammond, N. Formisano, P. Estrela, S. Carrara, J. Tkac, Electrochemical biosensors and nanobiosensors, Essays Biochem. 60 (2016) 69–80.
[129] S. Uniyal, R.K. Sharma, Technological advancement in electrochemical biosensor based detection of Organophosphate pesticide chlorpyrifos in the environment: a review of status and prospects, Biosens. Bioelectron. 116 (2018) 37–50.
[130] Y. Huang, J. Xu, J. Liu, X. Wang, B. Chen, Disease-related detection with electrochemical biosensors: a review, Sensors 17 (2017).
[131] T.H. Ho, F.X. Guillon, P. Bigey, F. Bedioui, M. Lazerges, Analysis of the evolution of the detection limits of electrochemical nucleic acid biosensors II, Anal. Bioanal. Chem. 409 (2017) 4335–4352.
[132] D. Grieshaber, R. Mackenzie, J. Vörös, E.J.S. Reimhult, Electrochemical biosensors - sensor principles and architectures, Sensors 8 (2008) 1400–1458.
[133] X. Zhou, S. Guo, J. Gao, J. Zhao, S. Xue, W. Xu, Glucose oxidase-initiated cascade catalysis for sensitive impedimetric aptasensor based on metal-organic frameworks functionalized with Pt nanoparticles and hemin/G-quadruplex as mimicking peroxidases, Biosens. Bioelectron. 98 (2017) 83–90.
[134] X. Zhou, S. Xue, P. Jing, W. Xu, A sensitive impedimetric platform biosensing protein: insoluble precipitates based on the biocatalysis of manganese(III) meso-tetrakis (4-N-methylpyridiniumyl)-porphyrinin in HCR-assisted dsDNA, Biosens. Bioelectron. 86 (2016) 656–663.
[135] H. Shu, W. Wen, H. Xiong, X. Zhang, S. Wang, Novel electrochemical aptamer biosensor based on gold nanoparticles signal amplification for the detection of carcinoembryonic antigen, Electrochem. Commun. 37 (2013) 15–19.
[136] P. Wang, Y. Wan, S. Deng, S. Yang, Y. Su, C. Fan, A. Aldalbahi, X. Zuo, Aptamer-initiated on-particle template-independent enzymatic polymerization (aptamer-OTEP) for electrochemical analysis of tumor biomarkers, Biosens. Bioelectron. 86 (2016) 536–541.
[137] J. Xiang, X. Pi, X. Chen, L. Xiang, M. Yang, H. Ren, X. Shen, N. Qi, C. Deng, Integrated signal probe based aptasensor for dual-analyte detection, Biosens. Bioelectron. 96 (2017) 268–274.
[138] M. Mazloum-Ardakani, Z. Tavakolian-Ardakani, N. Sahraei, S.M. Moshtaghioun, Fabrication of an ultrasensitive and selective electrochemical aptasensor to detect carcinoembryonic antigen by using a new nanocomposite, Biosens. Bioelectron. 129 (2019) 1–6.
[139] J.Y. Huang, L. Zhao, W. Lei, W. Wen, Y.J. Wang, T. Bao, H.Y. Xiong, X.H. Zhang, S.F. Wang, A high-sensitivity electrochemical aptasensor of carcinoembryonic antigen based on graphene quantum dots-ionic liquid-nafion nanomatrix and DNAzyme-assisted signal amplification strategy, Biosens. Bioelectron. 99 (2018) 28–33.
[140] Y. Zhao, Y. Yang, Y. Sun, L. Cui, F. Zheng, J. Zhang, Q. Song, C. Xu, Shell-encoded Au nanoparticles with tunable electroactivity for specific dual disease biomarkers detection, Biosens. Bioelectron. 99 (2018) 193–200.
[141] H. Cheng, L. Xu, H. Zhang, A. Yu, G. Lai, Enzymatically catalytic signal tracing by a glucose oxidase and ferrocene dually functionalized nanoporous gold nanoprobe for ultrasensitive electrochemical measurement of a tumor biomarker, The Analyst 141 (2016) 4381–4387.
[142] H. Quan, C. Zuo, T. Li, Y. Liu, M. Li, M. Zhong, Y. Zhang, H. Qi, M. Yang, Electrochemical detection of carcinoembryonic antigen based on silver nanocluster/horseradish peroxidase nanocomposite as signal probe, Electrochim. Acta 176 (2015) 893–897.
[143] Z. Liu, Y. Wang, Y. Guo, C. Dong, Label-free electrochemical aptasensor for carcino-embryonic antigen based on ternary nanocomposite of gold nanoparticles, hemin and graphene, Electroanalysis 28 (2016) 1023–1028.
[144] J.W. Park, W. Na, J. Jang, One-pot synthesis of multidimensional conducting polymer nanotubes for superior performance field-effect transistor-type carcinoembryonic antigen biosensors, RSC Adv. 6 (2016) 14335–14343.
[145] L. Ge, W. Wang, T. Hou, F. Li, A versatile immobilization-free photoelectrochemical biosensor for ultrasensitive detection of cancer biomarker based on enzyme-free cascaded quadratic amplification strategy, Biosens. Bioelectron. 77 (2016) 220–226.
[146] W. Deng, L. Shen, X. Wang, C. Yang, J. Yu, M. Yan, X. Song, Using carbon nanotubes-gold nanocomposites to quench energy from pinnate titanium dioxide nanorods array for signal-on photoelectrochemical aptasensing, Biosens. Bioelectron. 82 (2016) 132–139.
[147] X. Zeng, S. Ma, J. Bao, W. Tu, Z. Dai, Using graphene-based plasmonic nanocomposites to quench energy from quantum dots for signal-on photoelectrochemical aptasensing, Anal. Chem. 85 (2013) 11720–11724.
[148] Z. Qiu, J. Shu, D. Tang, $NaYF_4$:Yb, Er Upconversion nanotransducer with in situ fabrication of Ag_2S for near-Infrared light responsive photoelectrochemical biosensor, Anal. Chem. 90 (2018) 12214–12220.
[149] Z. Qiu, J. Shu, D. Tang, Near-infrared-to-ultraviolet light-mediated photoelectrochemical aptasensing platform for cancer biomarker based on core-shell $NaYF_4$:Yb,Tm@TiO_2 upconversion microrods, Anal. Chem. 90 (2018) 1021–1028.
[150] Z. Qiu, J. Shu, J. Liu, D. Tang, Dual-channel photoelectrochemical ratiometric aptasensor with up-converting nanocrystals using spatial-resolved technique on homemade 3D printed device, Anal. Chem. 91 (2) (2018) 1260–1268.
[151] K.S. Shin, J.H. Ji, K.S. Hwang, S.C. Jun, J.Y. Kang, Sensitivity enhancement of bead-based electrochemical impedance spectroscopy (BEIS) biosensor by electric field-focusing in microwells, Biosens. Bioelectron. 85 (2016) 16–24.
[152] G. Liang, Y. Man, X. Jin, L. Pan, X. Liu, Aptamer-based biosensor for label-free detection of ethanolamine by electrochemical impedance spectroscopy, Anal. Chim. Acta 936 (2016) 222–228.
[153] H. Li, X. Liu, L. Li, X. Mu, R. Genov, A.J. Mason, CMOS electrochemical instrumentation for biosensor microsystems: a review, Sensors 17 (2016).
[154] R. Elshafey, A.C. Tavares, M. Siaj, M. Zourob, Electrochemical impedance immunosensor based on gold nanoparticles-protein G for the detection of cancer marker epidermal growth factor receptor in human plasma and brain tissue, Biosens. Bioelectron. 50 (2013) 143–149.
[155] E.B. Bahadir, M.K. Sezginturk, A review on impedimetric biosensors, Artif Cells Nanomed Biotechnol 44 (2016) 248–262.
[156] E.P. Randviir, C.E. Banks, Electrochemical impedance spectroscopy: an overview of bioanalytical applications, Analytical Methods 5 (2013).
[157] A. Hayat, G. Catanante, J. Marty, Current trends in nanomaterial-based amperometric biosensors, Sensors 14 (2014) 23439–23461.
[158] J. Rick, M.C. Tsai, B.J. Hwang, Biosensors incorporating bimetallic nanoparticles, Nanomaterials (2015) 6.
[159] S. Azzouzi, L. Rotariu, A.M. Benito, W.K. Maser, M. Ben Ali, C. Bala, A novel amperometric biosensor based on gold nanoparticles anchored on reduced graphene oxide for sensitive detection of l-lactate tumor biomarker, Biosens. Bioelectron. 69 (2015) 280–286.
[160] W. Zhou, X. Gao, D. Liu, X. Chen, Gold nanoparticles for in vitro diagnostics, Chem. Rev. 115 (2015) 10575–10636.
[161] S.G. Meirinho, L.G. Dias, A.M. Peres, L.R. Rodrigues, Voltammetric aptasensors for protein disease biomarkers detection: a review, Biotechnol. Adv. 34 (2016) 941–953.
[162] F.S. Felix, L. Angnes, Electrochemical immunosensors - a powerful tool for analytical applications, Biosens. Bioelectron. 102 (2018) 470–478.
[163] E.B. Bahadir, M.K. Sezginturk, Poly (amidoamine) (PAMAM): an emerging material for electrochemical bio(sensing) applications, Talanta 148 (2016) 427–438.
[164] E.B. Bahadir, M.K. Sezginturk, Electrochemical biosensors for hormone analyses, Biosens. Bioelectron. 68 (2015) 62–71.
[165] V. Perumal, U. Hashim, Advances in biosensors: principle, architecture and applications, J. Appl. Biomed. 12 (2014) 1–15.
[166] A. Kim, C.S. Ah, C.W. Park, J.H. Yang, T. Kim, C.G. Ahn, S.H. Park, G.Y. Sung, Direct label-free electrical immunodetection in human serum using a flow-through-apparatus approach with integrated field-effect transistors, Biosens. Bioelectron. 25 (2010) 1767–1773.
[167] Z. Bao, J. Sun, X. Zhao, Z. Li, S. Cui, Q. Meng, Y. Zhang, T. Wang, Y. Jiang, Top-down nanofabrication of silicon nanoribbon field effect transistor (Si-NR FET) for carcinoembryonic antigen detection, Int. J. Nanomed. 12 (2017) 4623–4631.
[168] J. Li, G. He, H. Ueno, C. Jia, H. Noji, C. Qi, X. Guo, Direct real-time detection of single proteins using silicon nanowire-based electrical circuits, Nanoscale 8 (2016) 16172–16176.
[169] W.W. Zhao, J.J. Xu, H.Y. Chen, Photoelectrochemical enzymatic biosensors, Biosens. Bioelectron. 92 (2017) 294–304.
[170] H. Wang, Y. Wang, Y. Zhang, Q. Wang, X. Ren, D. Wu, Q. Wei, Photoelectrochemical immunosensor for detection of carcinoembryonic antigen based on 2D TiO_2 nanosheets and carboxylated graphitic carbon nitride, Sci. Rep. 6 (2016) 27385.
[171] J. Li, Y. Zhang, X. Kuang, Z. Wang, Q. Wei, A network signal amplification strategy of ultrasensitive photoelectrochemical immunosensing carcinoembryonic antigen based on CdSe/melamine network as label, Biosens. Bioelectron. 85 (2016) 764–770.
[172] W.W. Zhao, J.J. Xu, H.Y. Chen, Photoelectrochemical DNA biosensors, Chem. Rev. 114 (2014) 7421–7441.
[173] X. Zeng, J. Bao, M. Han, W. Tu, Z. Dai, Quantum dots sensitized titanium dioxide decorated reduced graphene oxide for visible light excited photoelectrochemical biosensing at a low potential, Biosens. Bioelectron. 54 (2014) 331–338.
[174] J. Wang, Y. Cao, Y. Xu, G. Li, Colorimetric multiplexed immunoassay for sequential detection of tumor markers, Biosens. Bioelectron. 25 (2009) 532–536.
[175] L. Tian, L. Liu, Y. Li, Q. Wei, W. Cao, Ultrasensitive sandwich-type electrochemical immunosensor based on trimetallic nanocomposite signal amplification strategy for the ultrasensitive detection of CEA, Sci. Rep. 6 (2016) 30849.
[176] L. Pan, J. Zhao, Y. Huang, S. Zhao, Y.M. Liu, Aptamer-based microchip electrophoresis assays for amplification detection of carcinoembryonic antigen, Clin. Chim. Acta 450 (2015) 304–309.
[177] C. Zong, J. Wu, M. Liu, L. Yang, F. Yan, H. Ju, Chemiluminescence imaging for a protein assay via proximity-dependent DNAzyme formation, Anal. Chem. 86 (2014) 9939–9944.
[178] Q. Fu, Z. Wu, F. Xu, X. Li, C. Yao, M. Xu, L. Sheng, S. Yu, Y. Tang, A portable smart phone-based plasmonic nanosensor readout platform that measures transmitted light intensities of nanosubstrates using an ambient light sensor, Lab Chip 16 (2016) 1927–1933.
[179] G.Y. Shen, H. Wang, T. Deng, G.L. Shen, R.Q. Yu, A novel piezoelectric immunosensor for detection of carcinoembryonic antigen, Talanta 67 (2005) 217–220.
[180] J.W. Lan, I.Y. Huang, Y.C. Lin, C.Y. Lin, J.L. Chen, C.H. Hsieh, Development of an FPW biosensor with low insertion loss and high fabrication yield for detection of carcinoembryonic antigen, Sensors 16 (2016).

[181] F. Zhou, M. Wang, L. Yuan, Z. Cheng, Z. Wu, H. Chen, Sensitive sandwich ELISA based on a gold nanoparticle layer for cancer detection, Analyst 137 (2012) 1779–1784.
[182] S. Lee, S.K. Chakkarapani, E.S. Yeung, S.H. Kang, Direct quantitative screening of influenza A virus without DNA amplification by single-particle dual-mode total internal reflection scattering, Biosens. Bioelectron. 87 (2017) 842–849.
[183] S. Ahn, H. Yu, S.H. Kang, Enhanced detection sensitivity of carcinoembryonic antigen on a plasmonic nanoimmunosensor by transmission grating-based total internal reflection scattering microscopy, Biosens. Bioelectron. 96 (2017) 159–166.
[184] P. Chen, K. Huang, R. Dai, E. Sawyer, K. Sun, B. Ying, X. Wei, J. Geng, Sensitive CVG-AFS/ICP-MS label-free nucleic acid and protein assays based on a selective cation exchange reaction and simple filtration separation, Analyst 144 (2019) 2797–2802.