Review

Discovery Medicine 2024; 36(186): 1316–1333
https://doi.org/10.24976/Discov.Med.202436186.122

Nanomaterial-Mediated Immunosensors and Their Performance in Detecting Tumor Markers

Yanping Xing[1], Xianli Lan[1,2], Xiyu Liu[1], Yong Huang[1,*]

[1]State Key Laboratory of Targeting Oncology, National Center for International Research of Bio-targeting Theranostics, Guangxi Key Laboratory of Bio-targeting Theranostics, Collaborative Innovation Center for Targeting Tumor Diagnosis and Therapy, Guangxi Medical University, 530021 Nanning, Guangxi, China
[2]School of Pharmacy, Guangxi Medical University, 530021 Nanning, Guangxi, China
*Correspondence: huangyong503@126.com (Yong Huang)
Published: 20 July 2024

The detection of tumor markers is crucial for assessing the progression of specific cancers. Numerous research studies have shown that immunosensors can convert immune-specific response biosignals into visual signals, enabling the highly sensitive tracking and detection of tumor markers. This offers a promising solution for early cancer diagnosis. However, most tumor markers are inert molecules that are challenging to detect at low concentrations in the early stages of cancer. Therefore, there is a need to develop immunosensor analysis platforms with a higher sensitivity. Nanomaterials, with their advantages of high stability, low cost, and versatility in design, have emerged as ideal candidates for enhancing the performance of immunosensor analysis. In this paper, we review the design ideas of nanomaterials in antibody-based electrochemical, electrochemiluminescent, and photoelectrochemical immunosensors, including electrode interface modification, signaling probes for stimulating sensing signals, and design strategies of modified materials in signaling mechanisms. In addition, we have thoroughly analyzed the performance, advantages and disadvantages of different immunosensors. Therefore, the aim of this paper is to review the recent advances in advanced nanomaterial strategies for different immunosensors and their biomedical applications, and to point out the challenges and prospects of immunosensors in future clinical applications.

Keywords: immunosensor; amplification strategies; construction mechanism; tumor marker; nanomaterials

Introduction

Cancer is a global challenge, with its complex and variable pathological processes and high metastatic and spreading power leading to high mortality rates among those who suffer from it [1]. It has been reported that 17 million people are expected to die from cancer each year by 2030 [2]. These data remind us of the urgency of improving the rapid diagnosis of cancer and finding effective treatments. Currently, the clinical diagnostic methods for cancer include imaging, pathological tissue biopsy, and tumor marker testing [3]. Undoubtedly, tissue biopsy is still the gold standard for clinical cancer diagnosis [4]. However, this method is risky and painful for patients and has low sensitivity. In contrast, the detection of tumor markers has become a reliable tool for early cancer diagnosis due to its non-invasive means and disease-specific advantages [5]. Tumor markers are biomolecules that are expressed abnormally by tumor cells or normal cells in various body tissues and fluids, such as blood, urine, and cerebrospinal fluid. The presence or absence of these markers in the body and their levels are closely associated with the development of diseases [6]. For example, glycoprotein alpha-fetoprotein (AFP), which has a high concentration in fetal circulation, is a normal phenomenon, and the concentration gradually decreases after birth. It is difficult to detect, and if a high concentration of AFP is detected in adult serum, then this suggests that there is a possibility of hepatocellular carcinoma (HCC) [7]. With the developments in life sciences and technology, the clinical value of a tumor marker is no longer confined to the aspect of auxiliary diagnosis. The changes in the serum concentration of these markers are now closely linked to the assessment of tumor efficacy, tumor recurrence, prognosis, and treatment. In particular, when guiding clinical targeted drug use, the precise detection of tumor markers is conducive to making the best choice regarding tumor treatment plans [8]. Thus, there is a need for the sensitive detection of tumor markers.

To date, various established techniques have been utilized in clinical laboratories for the detection of tumor markers, including the enzyme-linked immunosorbent assay, radioimmunoassay, chemiluminescence, and biosensor assay [9–11]. These methods can meet the needs of clinical diagnosis and treatment monitoring to a certain extent, but it is difficult to detect serum tumor markers in the early stages of cancer, leading to most cancers already having metastasized by the time they are detected, with a poor therapeutic prognosis. In order to solve this problem, a va-

riety of low-limit, wide-range detection methods have been explored, among which biosensors stand out for their high sensitivity, simplicity, and rapidity. A biosensor is a small device that combines the specificity of the recognition element and the sensitivity of the transducer to obtain visual information from signal amplification [12]. Since the creation of the first biosensor in 1956, a large number of biosensors have been developed based on its detection principle [13]. There are more than 900 literature retrieval results from the last ten years with the themes of "biosensor" and "tumor markers" in the Web of Science database, and researchers are constantly exploring them to find better methods to apply to the detection and analysis of tumor markers.

In order to meet the needs of clinical health analysis, sensitive element antibody-based immunobiosensors have been widely used in disease diagnosis and tumor therapy monitoring due to their immunoassay specificity and the sensitivity of their sensing techniques [14]. The biological signals of immune reactions are typically presented as electrical signals, optical signals, and photocurrent conversions, depending on the different signal transduction mechanisms. This further divides immunosensors into electrochemical, electrochemical luminescence, and photoelectrochemical immunosensors [15]. Among these, the electrochemical immunosensor remains the most widely used and the classic immunosensor due to its high detection efficiency and low cost, and due to there being no need for auxiliary photosensitive materials [16]. In particular, its high selectivity and level of convenience render it an ideal device for immediate detection at the bedside at nurse checks [17]. As a combination of chemiluminescence and electrochemistry, electrochemiluminescence combines the advantages of the two methods, and it is a new technology for immunosensor analysis [18]. Unlike other optical sensors, an electrochemiluminescence immunosensor does not need additional light sources or fluorescent reagents, so its anti-interference ability is remarkable [19]. Compared to the previous two, the unique advantages of photoelectrochemical immunosensors, with the separation of excitation light sources and detection signals, low background signals, and low potentials, overcome the drawbacks of low photoconversion efficiencies and photobleaching effects found in some conventional sensor analysis platforms [20]. The advantages and disadvantages of electrochemical, electrochemiluminescent, and photoelectrochemical immunosensors are shown in Table 1 (Ref. [16,18,20]).

In terms of clinical significance in disease diagnosis, low detection limits play a crucial role in disease prevention and treatment. Sensor devices can enhance the signal strength [21]. Thus, various signal amplification technologies for immunosensors have emerged. Strategies like the rolling amplification reaction, enzyme cascade reaction, and hybrid chain reaction can quickly amplify small biological signals step by step and ultrasensitively analyze tumor markers in blood samples. However, the reaction process is affected by factors such as the temperature, pH, and operating conditions, and further optimization of the conditions is required for practical clinical applications [22]. In contrast, a variety of functionalized nanomaterials are rapidly attracting the attention of researchers due to their stable properties and ease of manipulation, and there is great potential for the bioanalysis of nanomaterials designed individually or synergistically with other means through additional technology [23].

At present, most of the reviews are dominated by the application of nanomaterial classification in antibody immunosensors [24,25]. And, based on the principle of the signaling mechanism of antibody immunosensors, the active selection and clever design of nanomaterials are equally important for the construction mode and signal enhancement of immunosensors (Fig. 1). Therefore, the core of this review is based on the sensing mechanisms of electrochemical, electrochemiluminescent, and photoelectrochemical immunosensors, describes the recent research progress in the design of nanomaterial sensitivity enhancement strategies, and discusses the analytical performance of immunosensors developed using nanomaterials strategies. Finally, it provides insights into the future development of nanomaterial technology for the diagnosis and treatment of clinical diseases.

Electrochemical Immunosensor

Electrochemical immunosensors (ECs) aim to convert the small signals generated through the interaction of fixed biomolecules with target substances into more easily interpretable electrical signal changes [26]. These changes are typically observed in the form of current signal changes resulting from the oxidation-reduction reaction of the electroactive substance acting as a signal probe or the impedance changes in the sensor after capturing insulating biomolecules [27], and are detected through cyclic voltammetry (CV), square wave voltammetry (SWV), differential pulse voltammetry (DPV), and other electrochemical techniques [28]. In order to amplify these detection signals, researchers have introduced nanomaterials for the design of ideal detection devices that are based mainly on two construction modes: label-free and sandwich. Among them, during label-free electrochemical immunosensor sensitivity amplification, the primary consideration is improving the electrochemical signal response value by directly modifying the electrodes with nanomaterials as the substrate material for the quantitative detection of the target analytes. Unlike the unlabeled immunosensors, the sandwich form of electrochemical immunosensors analyzes antigen-antibody interactions in terms of interfacial responses triggered by nanomaterial signal probes. Therefore, in the following sections, we will detail advanced strategies for nanomaterial sensitivity amplification in both electrochemical sensor construction modes and present our insights.

Table 1. The advantages and disadvantages of electrochemical, electrochemiluminescent, and photoelectrochemical immunosensors.

Immunosensors	Advantages	Disadvantages	Ref.
Electrochemical	Low cost, rapid and sensitive detection, simple equipment, wide range of application	Stability is not good enough, weak anti-interference ability	[16]
Electrochemiluminescence	Strong sensitivity, good anti-interference ability, good stability, wide analysis range	Single-signal output systems are susceptible to external conditions, resulting in low accuracy and repeatability	[18]
Photoelectrochemical	Low background signal, continuous detection, fast analysis, good reproducibility	Light may deplete biomolecular activity, resulting in unsatisfactory assay results	[20]

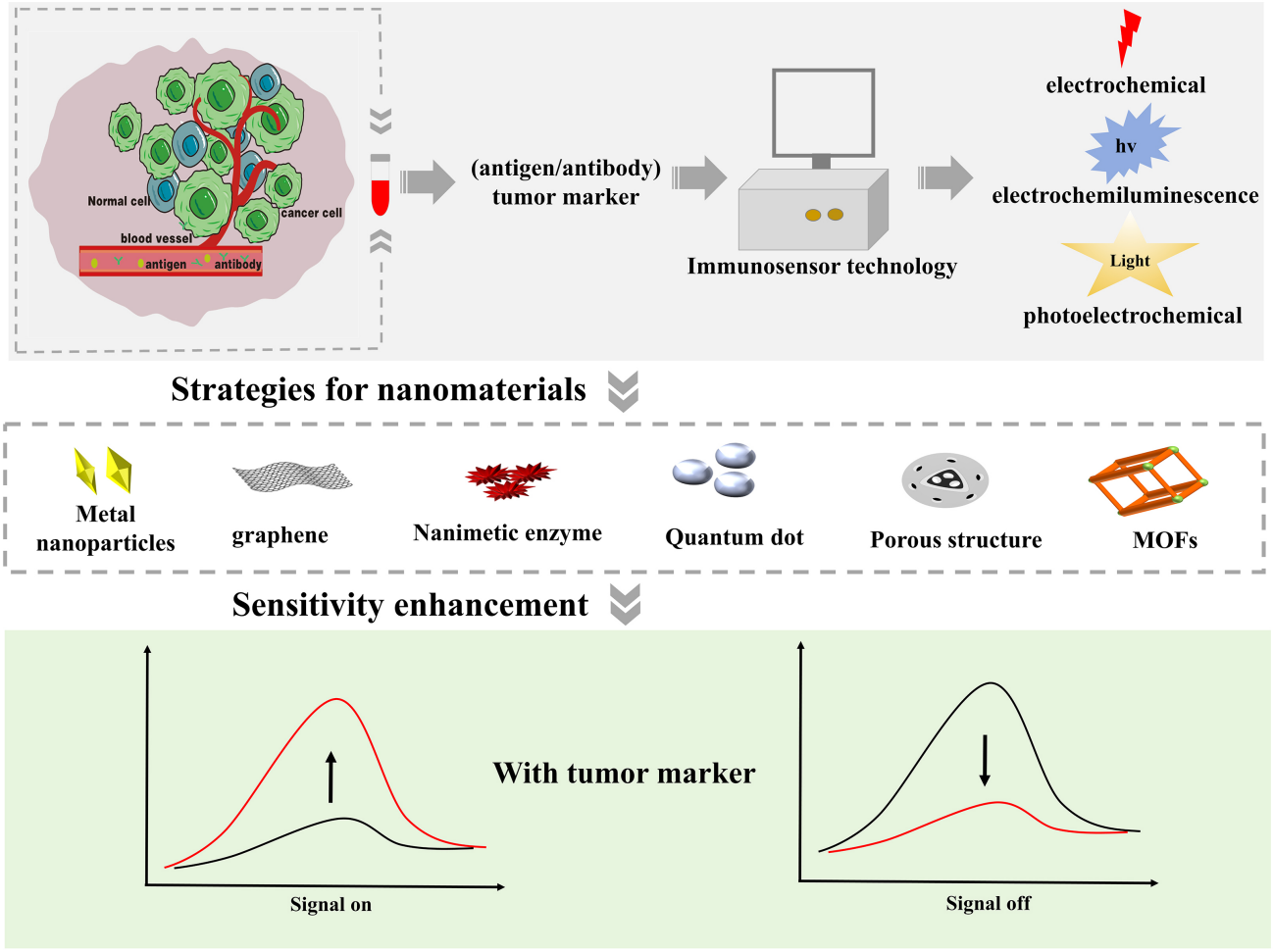

Fig. 1. Schematic overview of immunosensors. Electrochemical, electrochemiluminescence, and photoelectrochemical immunosensor methods are used to detect tumor marker antigens or antibodies in biological samples. Nanomaterials are cleverly designed based on the principles of various types of immunosensors. Specifically, through strategies such as electrode modification, high-signal label construction, and nanomaterial modifications that allow direct action on the signal transduction mechanism, the immunoassay results show that the sensitivity is significantly enhanced. The figure was created using Adobe Illustrator 2019 (Adobe, San Jose, CA, USA) and Microsoft Office 2016 (Microsoft, Redmond, WA, USA). MOFs, metal-organic frameworks.

Substrate Nanomaterials Promote Electrochemical Reactions

In label-free immunoassays, the modification of electrodes by substrate nanomaterials allows for changes in electrochemical signals, such as the electrode surface potential and electron transfer rate before and after the immunoreaction event, and the concentration and conductivity of target molecules on the electrode surface are key factors affecting the final signal response [29]. Considering their conductivity, gold nanoparticles (Au NPs) are mentioned in a large number of studies. Metal nanoparticles have good conductivity and biocompatibility, which are benefi-

cial to the immobilization of antigens (antibodies) on the electrode surface [30]. For this reason, a large number of precious metal nanoparticles (such as platinum, gold, silver, and palladium) or composite metal nanomaterials have recently been applied to the construction of electrochemical (EC) immunosensors [31]. The strong conductivity of a metal material is beneficial to the electron transfer rate on the electrode surface, and it can also self-assemble with biomolecules to form a stable substrate structure, thus improving the stability of the immunosensor [32].

In order to increase the specific surface area of the electrode and enhance the biomolecule enrichment, some two-dimensional carbon-based nanomaterials with a large surface area and good electrical conductivity have been introduced. Like precious metal materials, carbon-based materials are extensively utilized in cancer marker detection, the targeted delivery of tumor drugs, and cancer monitoring [33]. Carbon-based nanomaterials provide technical support for the quantitative detection of protein molecules through the use of either individual carbon materials or modified composite carbon-based nanomaterials as modified electrode substrates [34]. Graphene is a two-dimensional carbon nanomaterial with good conductivity and strong mechanical properties and can be physically adsorbed with biomolecules through π-π bonds without losing the biological activity of the molecules [35]. However, it is found that the simple physical adsorption in an immunoassay cannot meet the requirements of highly sensitive detection of immunosensors. Therefore, a method of modifying graphene with highly conductive materials is proposed [36], such as using gold-platinum alloy modified boron-doped graphene nanosheets (AuPt-BG) as the base material to construct an immunoassay platform for detecting glycoprotein antigen 153 (CA153). Different from pure graphene, boron-doped graphene nanoplates have a 3D porous structure and different charge distribution regions, which can generate two-channel redox reactions in an electrolyte solution at the same time [37]. With the cooperation of metal nanoparticles, the signal response of the chemical reaction on the electrode surface is stronger [38]. Additionally, the highly controllable characteristics of AuPt-BG also provide a new method for preparing flexible microelectrodes *in vivo* to monitor the concentration of tumor markers *in vivo* [39]. Although graphene has great application value, it is easily oxidized into oxidized graphene. Oxidized graphene damages the properties of graphene itself and inevitably leads to partial loss [40]. Therefore, a design for graphene allotrope carbon nanomaterial single-walled carbon nanoangle (SWCNH) and nitrogen-doped graphene quantum dot hybrid modified electrodes is proposed. The constructed EC immunosensor can perform sensitive monitoring of AFP, exhibiting a wide detection range from 0.001 ng mL^{-1} to 200 ng mL^{-1} and a detection limit as low as 0.25 pg mL^{-1} under optimal conditions [41]. SWCNHs help prevent the aggregation of nanomaterials on the electrode, while nitrogen-doped graphene quantum dots enhance the surface conductivity and offer stronger selectivity for target molecules compared to other carbon-based materials [42]. Therefore, the construction of target biomolecular enrichment substrate materials with excellent electrochemical analysis performance using graphene-based composite nanomaterials and their allotropes is a simple and feasible signal enhancement strategy.

Currently, a nanocomposite with electrocatalytic properties is prepared by assembling quantum dots and metal nanoparticles, etc., to modify the electrode surface, and, after the immunoreaction, the substrate is added, and biomolecules are detected via electrochemical technique chronoamperometry (I-T), which results in a substantial increase in sensitivity compared with other label-free methods [43]. This strategy of promoting electrochemical reactions by enabling electrocatalytic reactions without the need for enzymatic catalysis via signal probes has great potential for the construction of highly sensitive electrochemical immunosensors. The schematic design of the sensitivity amplification strategy for different substrate nanomaterials is shown in Fig. 2.

In addition, a new label-free electrochemical immunosensor sensitivity enhancement technology has been developed, and some laboratories use chip electrodes constructed from electropolymers, quantum dots, and three-dimensional composite nanomaterials or branch electrodes for multi-tumor marker detection, prepared from special materials for the quantification of antigens, antibodies, and other tumor markers [44,45]. The constructed electrochemical immunoassay platforms have a significantly enhanced sensitivity. Martins' team [46] used an electrodeposition method to prepare immunosensor chips from gold dendritic nanostructures (AuDdrites), which are able to directly adsorb the bioassay and capture molecules without labeling to specifically bind to the molecules to be tested, making them an ideal tool for self-testing assays. Compared to labeled probe-promoted electrochemical reactions, the optimized materials are recommended for designing high-performance immunosensor chips because (I) the adopted hyperbranched dendritic structure materials have a large surface area and high electrical conductivity, which satisfy the two demands of label-free immunosensors and offer strong electrocatalytic activity; (II) the operation process is simple and convenient, without the need for a larger volume of biomaterials; and (III) this nanostructure can be designed for use in wearable sensors, making it promising nanomaterial.

Nanomaterial Signaling Labels Trigger Interfacial Reactions

Unlike label-free EC immunosensors, sandwich structure immunoassays have more room for manipulation in the amplification sensitivity strategy. Therefore, for sandwich electrochemical immunosensors, markers are intro-

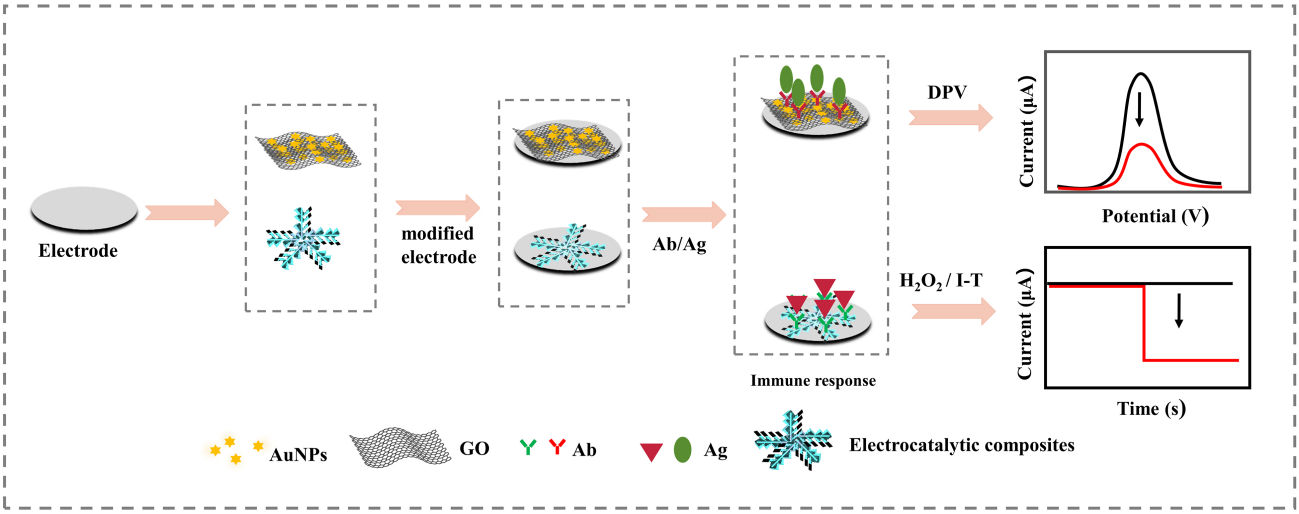

Fig. 2. Schematic diagram of the modified electrode strategy for substrate nanomaterials. The first approach synergistically modified the electrode using two types of nanomaterials with improved electrical conductivity and increased specific surface area, gold nanoparticles (Au NPs) and graphene (GO), and after capturing the target antigen (Ag), the electrochemical technique cyclic differential pulse voltammetry (DPV) assay showed a significant enhancement in sensitivity. The second method used electrocatalytically active nanocomposites to modify the electrode, and after the immunoreaction, the hydrogen peroxide (H_2O_2) substrate was added and detected using electrochemical chronoamperometry (I-T) technique, which showed that the substrate material had excellent catalytic performance and significant electrochemical signal amplification. The figure was created using Microsoft Office 2016 (Microsoft, Redmond, WA, USA).

duced to label the antigen/antibody as a signal probe, and when the immune specific binding reaction is achieved, the tracking technology of the signal probe and its signal amplification successfully amplify the signal of low-abundance tumor markers for quantitative detection. The first strategy is that porous nanomaterials with multiple binding sites act as carriers for signaling substances, such as common mesoporous silicon, organic framework materials, layered graphene, and porous carbon spheres [47]. The rich pore structure enriches a large number of electroactive substances and can efficiently immobilize biomolecules, so that with the generation of the immune-reaction complexes method, the concentration of the target tumor markers and the intensity of electrochemical signals generated by the electroactive substances show a linear relationship to achieve a signal amplification effect [48]. This signaling-molecule enrichment provides a potential design idea for electrochemical reaction signal amplification. Liang's team [49] used a porous covalent organic skeleton (COF) loaded with the electroactive substance thionine (Thi) to synthesize the high-signaling probe COFTFPB-Thi (an electroactive nanocomposite prepared by an amine-formaldehyde condensation reaction between the reagents TFPB and Thi). In this design scheme, AuNPs were introduced to construct a stable host-guest complex of AuNPs/COFTFPB Thi, which provides stable Au-S bonds that can bind to the secondary antibody. The constructed antibody tag has a dual signal amplification effect. The host-guest composite nanomaterials are loaded with specific reactive antibodies, and, ultimately, the host-guest substances, as signal molecules, are enriched in the biological binding event, synergistically enhancing the electrical signal response [50]. After the sandwich immune reaction occurs, the enhanced electrochemical signal achieves the ultrasensitive detection of carcinoembryonic antigen (CEA), with detection as low as 0.034 ng mL^{-1} (Fig. 3).

Enzyme-labeled antibodies act as signal probes to trigger electrochemical reactions, and electrochemical signal amplification occurs in enzymatic immunoreactions as another strategy to improve the sensitivity of sandwich electrochemical immunoassay platforms [51]. Traditionally, the natural enzyme horseradish peroxidase (HRP) or alkaline phosphatase (ALP) is used as the labeling enzyme, but, considering the influence of the working environmental conditions of electrochemical immunosensors, the activity of the natural enzyme is often affected to a certain extent [52], so, with the development of nanotechnology, many nanomaterials with a large surface area and excellent conductivity mimic the enzyme to offer an alternative to labeling the antibody with part of the natural enzyme. For example, Fang's team [53] used Au@ZnO with peroxidase-like catalytic activity to label antibodies with HRP simultaneously, and both of them cooperated in the electrocatalytic reaction to achieve the sensitive detection of the tumor marker AFP.

Differently from traditional methods, researchers try to improve the sensitivity of immunoanalysis by using labeled probes to catalyze a precipitation reaction to cause

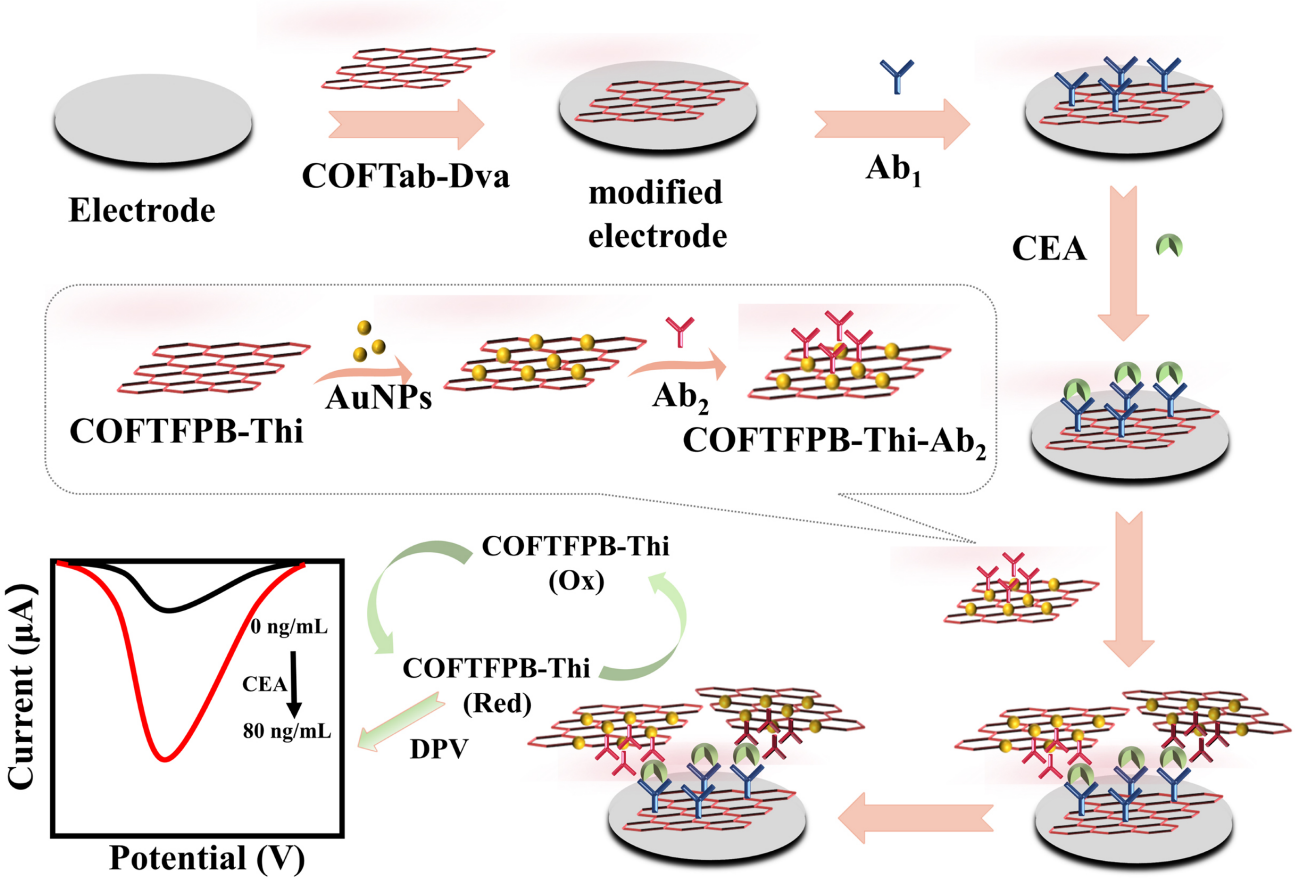

Fig. 3. Schematic diagram of host-guest recognition of enriched signal substances mediated by gold nanoparticles. Gold nanoparticles (Au NPs)-mediated host-guest technique assembles the secondary antibody (Ab_2) with the high-signal marker complex COFTFPB Thi, while synergizing with the porous organic skeleton of the modified electrode to promote the electrocatalytic reaction. The figure was created using Microsoft Office 2016 (Microsoft, Redmond, WA, USA). Thi, thionine; CEA, carcinoembryonic antigen.

a current difference. However, this design, similar to impedance changes, has high requirements for background interference signal processing and is not ideal for future clinical research. Therefore, benefiting from the inspiration of electrocatalytic precipitation reactions, sensors in which enzyme-labeled probes catalyze the generation of electroactive substance precipitates to achieve signal enhancement continue to be at the forefront of researchers' minds. A method is proposed based on tyramine signal amplification (TSA) technology to catalyze the electroactive molecule ferrocene-tyramine (Fc-Tyr) deposition reaction to achieve signal amplification [54]. In this method, magnetic nanomaterials are used as an immune reaction platform. After the enzyme-loaded magnetic bead biological binding product system is dripped on the screen-printed electrode modified with tyramine, the enzyme triggers the TSA reaction to induce a large number of Fc-Tyr to deposit on the electrode surface. The strong redox ability of Fc-Tyr allows the sensitive detection of the liver cancer marker Glypican3 (GPC3). The use of magnetic nanoparticles helps minimize matrix effects, while the presence of free electroactive substances enhances electrocatalytic activity [55]. This design of a quasi-homogeneous system with synergistic electrocatalytic reactions has gained popularity in the development of EC immunosensors for detecting tumor markers. Thus, the electrocatalytic precipitation reaction enriches electroactive molecules with high sensitivity for amplifying sandwich-type immunoassays.

In addition, the third sandwich electrochemical immunosensor signal probe to improve the sensitivity of the immunoassay strategy is based on the molecular biology of nucleic acid amplification technology, such as rolled-circle amplification (RCA), polymerization chain reaction (PCR), and hybridization chain reaction (HCR) [56]. At first, nucleic acid amplification technology (PCR technology) based on enzyme-linked immunosorbent assay (ELISA) was used in immunoassay [57]; however, with the continuous improvements in technology, the RCA method, breaking the PCR technology's thermal cycling conditions, began to be applied to the design of sensors. RCA's high amplification capacity and stability allow it to be utilized for primers for a large number of loaded active molecules in the sensitive element, so that the antibody immunoassay can achieve the results seen in the study [58]. The RCA method

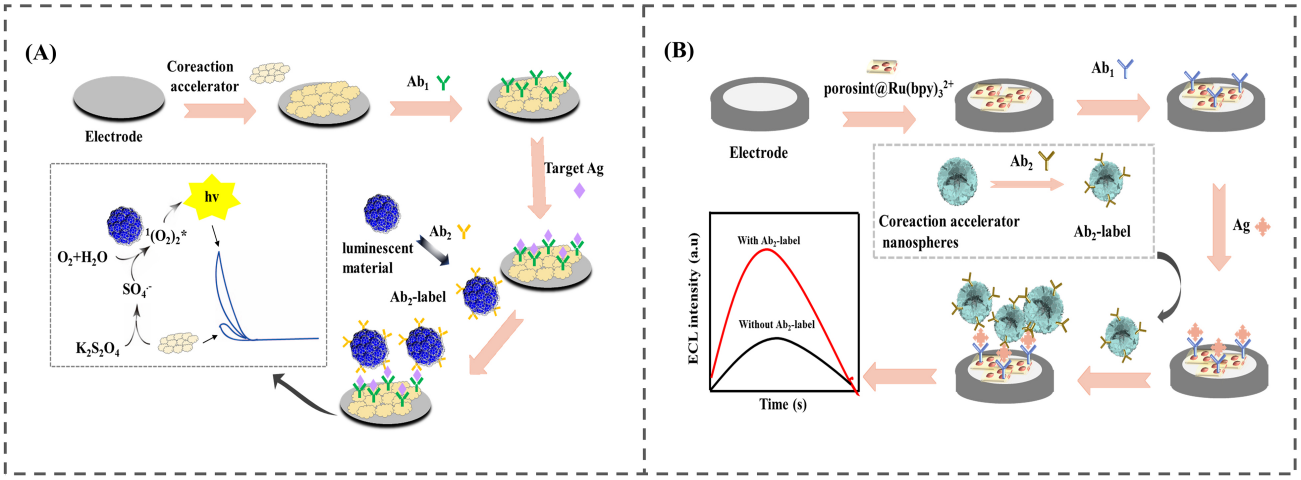

Fig. 4. Single-signal output electrochemiluminescence (ECL) immunosensor. (A) The composite nanomaterial CuS/HPC acts as a co-reaction promoter to modify the electrode and promotes the efficient conversion of $K_2S_2O_8$ into the luminescent active substance $SO_4^{\cdot-}$, which drives the enhancement of the ECL signal with the increase in the concentration of the target biomolecule. (B) Porous nanomaterials as nanoreactors encapsulating $Ru(bpy)_3^{2+}$ luminescent reagents synergistically enhance ECL signal intensity using the co-reaction promoter functionalized nanomaterials of labeled secondary antibodies. The figure was created using Autodesk 3ds Max (Autodesk, San Rafael, CA, USA) and Microsoft Office 2016 (Microsoft, Redmond, WA, USA). HPC, 2D high porous carbon. $Ru(bpy)_3^{2+}$, Tris(bipyridine)ruthenium(II) ion.

has a high sensitivity, but its high cost and the high requirements for the storage conditions of the related reagent mean that the use of the current experiments still needs to be considered. In addition, compared with the antigen-antibody detection immunoassay platform, molecular biology technology sensitivity enhancement technology in nucleic acid tumor markers is more widely used but is also the future of electrochemical immunoassay technology development.

Electrochemical Luminescence Immunosensor

The signal-sensing process of the electrochemiluminescence (ECL) immunosensor is that the substance on the electrode is stimulated by voltage, then forms an excited state after electron transfer, and then goes through the radiation relaxation process to output optical signals [59]. To date, more and more ECL systems have been put forward, and clinical laboratories have also applied this technology to the health analysis of clinical patients [60]. The electroluminescent efficiency of signal reagents is one of the factors affecting the performance analysis of immunosensors [61]. At present, a large number of light-emitting reagents have been reported and are mainly divided into organic small molecules, inorganic complexes, and nanomaterials, among which the classical and representative electrochemical luminescence light-emitting reagents are Luminol, Tris(bipyridine)ruthenium(II) ion ($Ru(bpy)_3^{2+}$) and quantum dots (QDs) [62,63]. With the continuous exploration of new nanomaterials, a series of high-performance electrochemical luminescence immunoassay platforms based on nanomaterials starting from quantum dots have gradually become the research focus. During electrochemiluminescence immunosensing, the rational design of nanomaterials in the sensing building mechanism improve the shortcomings of simple single-signal output ECL immunoassay devices with low sensitivity by improving the luminescence efficiency of signal reagents, due to the diversity of the test samples, the dual-signal output ECL immunosensors, which can be self-calibrated, have been investigated according to the high sensitivity single-signal detection mode. Similarly, nanomaterial strategies act according to their construction mechanisms to enhance the sensitivity of the test samples while greatly improving the reliability of the results. Therefore, the immunoassay qualities of both single-signal-output and dual-signal-output ECL immunosensors with nanomaterial strategies acting on them are described in detail in the following section, and their developmental prospects in clinical immunoassays are evaluated.

Nanomaterial-Based Single-Signal Output ECL Immunosensor

When the immunosensor is working, the ECL signal intensity shows a trend with the concentration of the target analyte to output a single signal, and the results are clear at a glance. In order to amplify the output signal, various nanotechnology strategies have been explored [64], among which, the method of introducing nanomaterial co-reaction promoters to provide active intermediates is expected to enhance the ECL immunosignal. Based on this, as shown in Fig. 4A, one study [65] used 2D high porous carbon (HPC) loaded with spherical nanoflower-like CuS to prepare the

co-reaction promoter composite CuS/HPC, which acts on the ECL immunoassay platform for detecting CA242. The highlight of this work is the reaction promoter CuS/HPC. From the perspective of enhancing ECL signals, CuS/HPC promotes the conversion efficiency of potassium persulfate ($K_2S_2O_8$) in the ECL system. It precisely provides the required $SO_4^{\bullet-}$ active substance for the dual-luminescence system of SnS_2 QDs and dissolved oxygen, and the good driving effect significantly enhances the ECL signal as the concentration of target biomolecules increases.

$Ru(bpy)_3^{2+}$ is a classic luminescent group in the ECL system, but it does not have functional groups and is easily soluble, making it difficult to convert it to being fixed directly on the sensor [66]. Therefore, a dual-signal amplification strategy using nanoreactors and co-reaction accelerators is considered to make up for this defect in the luminophore [67]. As shown in Fig. 4B, a porous material with stable chemical properties and porous properties acts as a nanoreactor to encapsulate the $Ru(bpy)_3^{2+}$ luminescent reagent. Compared to ordinary luminophores, the newly assembled ECL signaling reagent demonstrates exceptional luminescence efficiency and supports large amounts of antibody immobilization with abundant functional groups. The co-reaction accelerator introduced by the labeled secondary antibody can further enhance the ECL signal intensity. This sensing strategy enables the achievement of an ideal immunoanalysis of neuron-specific enolase (NSE) [68]. However, it cannot be ignored that during the ECL reaction process, there are inevitably problems such as a long electron conduction distance, energy loss, and excessive use of co-reaction accelerators [69]. Therefore, further exploration of more advantageous design solutions is warranted.

Compared with the co-reaction promoter, the researchers proposed a self-enhanced mode signal amplification strategy by linking co-reactants and luminescent reagents into a covalent nanocomposite via chemical bonding so that electron transfer would take place within the molecule, and this approach could overcome the problem of excessive co-reactive promoters in the ECL reaction system [70]. For example, Luo *et al*. [71] bridged the ECL luminescent material $Ru(bpy)_2(mcpbpy)^{2+}$ with the co-reactant polyethyleneimine (PEI) through chemical amide bonding, and the prepared ECL luminescent complex Ru-PEI was able to shorten the electron transfer distance between the luminescent cluster and the co-reactant to improve the efficiency of the luminescent cluster. In this study, the authors also immobilized the prepared self-enhanced complexes in the well-stabilized multibranched nanomaterials. Therefore, the constructed ECL immunoassay platform was able to achieve the sensitive detection of the cancer biomarker caspase-3 in the immunosensing process.

At present, the most classic luminescence mode is the combination of metal nanoclusters and co-reactants. Metal nanoclusters have a unique energy level structure and photoelectric properties, which can stably combine co-reactants [72]. When the ECL immunosensor works, the composite structure of emitter and co-reactants greatly shortens the transmission distance between them, which significantly enhances ECL efficiency and is widely used in tumor marker detection and tumor microenvironment small molecule detection [73]. The results of the immunoassay of ECL based on a nanotechnology design strategy show that introducing functionalized nanomaterials into the ECL sensing mechanism is a feasible sensitivity enhancement strategy.

Nanomaterial-Based Dual Signal Output ECL Immunosensor

Due to interference from external factors such as the environment and instruments, single-signal output ECL sensors are prone to false results [74]. Therefore, ratiometric dual-signal output mode ECL immunosensors have attracted the attention of researchers. Inspired by the principle of ratiometric fluorescence in the early days, Zhang's research group [75] developed a dual-potential signal ECL immune platform based on two CdS QD-luminol emitters for cancer gene detection. The introduced CdS nanocrystal (NC) showed excellent cathode ECL properties. In the presence of hydrogen peroxide (H_2O_2), after adding Pt nanoparticles (NPs) labeled biomolecules dropwise, it was found that Pt NPs have an efficient quenching ability for CdS NC and, at the same time, can enhance the ECL properties of luminol. This phenomenon varies in parallel with the ECL signal, and its ratio is linearly related to the concentration of the biomolecule to be measured. The team then developed another dual-wavelength resolution signal ECL immunosensor based on the electrochemiluminescence resonance energy transfer (ECL-RET) principle [76], and its sensing mechanism is shown in Fig. 5A. In order to achieve the overlapping condition between the energy donor and the energy acceptor, Au NPs were used to functionalize graphitic carbon nitride nanosheets composite (g-C_3N_4 NS) to prepare hybrid nanometers of the material Au-g-C_3N_4 NH. The ECL spectrum of this nanocomposite at a wavelength of 460 nm matches well with the $Ru(bpy)_3^{2+}$ absorption peak, producing efficient ECL-RET. When we analyze the design strategies for enhancing the sensitivity of two dual-signal ECL immunosensors constructed by the same team, we find that nanomaterials can amplify ECL signals through the coupling of the electrochemical reaction and energy transmission on the electrode surface. Therefore, it is key that we improve the sensitivity of the immunoassay to build a new nanomaterial ECL emitter with an enhanced ECL signal, higher stability, and lower toxicity on the sensitive detection platform for different cancers.

In addition to quantum dots and metal-functionalized semiconductor carbon materials, researchers have tried to combine metal-organic frameworks (MOFs) with luminescent reagents. On the one hand, MOFs prepared with organic ligands have excellent electrochemical luminescence

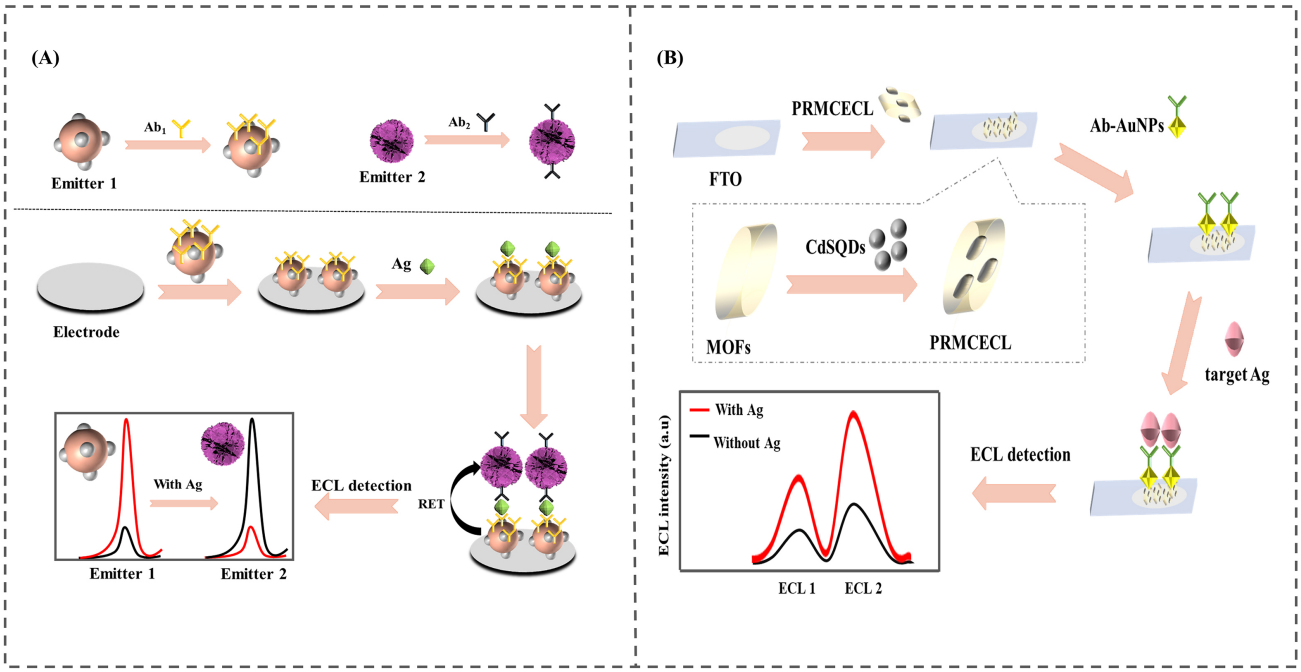

Fig. 5. Dual-signal output ECL immunosensor. (A) Schematic diagram of electrochemiluminescence resonance energy transfer (ECL-RET) sensing mechanism: Emitter 1 loaded with recognizing biomolecules is immobilized on the electrode, and the introduced hybridized nanomaterials of labeled secondary antibody of emitter 2 quench the signal of emitter 1, which together form a complete dual-signal output pattern. (B) Schematic diagram of a label-free differential ECL immunesensor: PRMCECL nanoluminescent clusters generate two potential signals in neutral solution. After capturing the target antigen, the charge distribution on the electrode surface of the immunosensor is interfered with by the antigen molecules resulting in weakened impedance, which leads to the amplification of the sensing signal. The figure was created using Autodesk 3ds Max (Autodesk, San Rafael, CA, USA) and Microsoft Office 2016 (Microsoft, Redmond, WA, USA). FTO, fluorine-doped tin oxide electrode.

properties and can be directly used as emitters. On the other hand, MOFs are stable, with a large specific surface area and a porous structure carrying a large number of luminescent reagents to enhance the emitter function [77]. In a recent study, a proportional ECL immunoassay platform was developed using PRMCECL nanoluminescent clusters prepared from MOF-5-encapsulated CdS QDs. These nanoluminescent clusters exhibited two potentials in a neutral aqueous solution. When the target antigen is captured, it leads to a change in the charge distribution on the surface of the immunosensor electrode and attenuates the impedance. The output signal is significantly augmented compared to the initial signal. The platform enables the quantitative detection of cardiac troponin I (cTnI) in the presence of the co-reactant $K_2S_2O_8$ by subtracting one of the intrinsic ECL signals from the overall ECL response signal [78] (Fig. 5B). However, the research on the electrochemiluminescence activity of MOFs is only in the primary stages, and the spatial distribution of the assembled composite molecules and its influence on the biomolecule activity need to be further explored.

It is well known that nanomaterial strategies for enhancing the sensitivity of ECL immunosensors introduce co-reactants in addition to optimizing the ECL properties of the emitter. In particular, it is now found that the electrode surface temperature has a certain effect on the ECL signal intensity [79], therefore, a series of system-heating solutions and the introduction of a large number of heat-producing nanomaterial probes and heat-producing indium tin oxide (ITO) electrodes to increase the electrode surface temperature have been applied to the design of ECL immunosensors [80]. For example, Fang *et al.* [81] screened a photothermal agent carbon nanohorn, with a large specific surface area and good photothermal properties, that acted as an anode emitter in the constructed proportional ECL immunosensor, which could efficiently perform energy conversion, increase the electrode surface temperature, and enhance the output ECL immunoassay signals, and offered characteristics for the detection of human epididymis protein 4 (HE4), a biomarker of ovarian cancer, with the low detection limit of 3.3×10^{-6} ng mL^{-1}.

While label-free differential ECL immunosensors and energy resonance transfer-based ECL ratio immunoassays are currently available, the application of ECL ratio-sensing analysis in clinical immunoassays is still in its early stages. One of the main challenges is designing suitable emitter nanomaterials. To broaden the clinical applications of ECL immunosensors, it is crucial to conduct further research

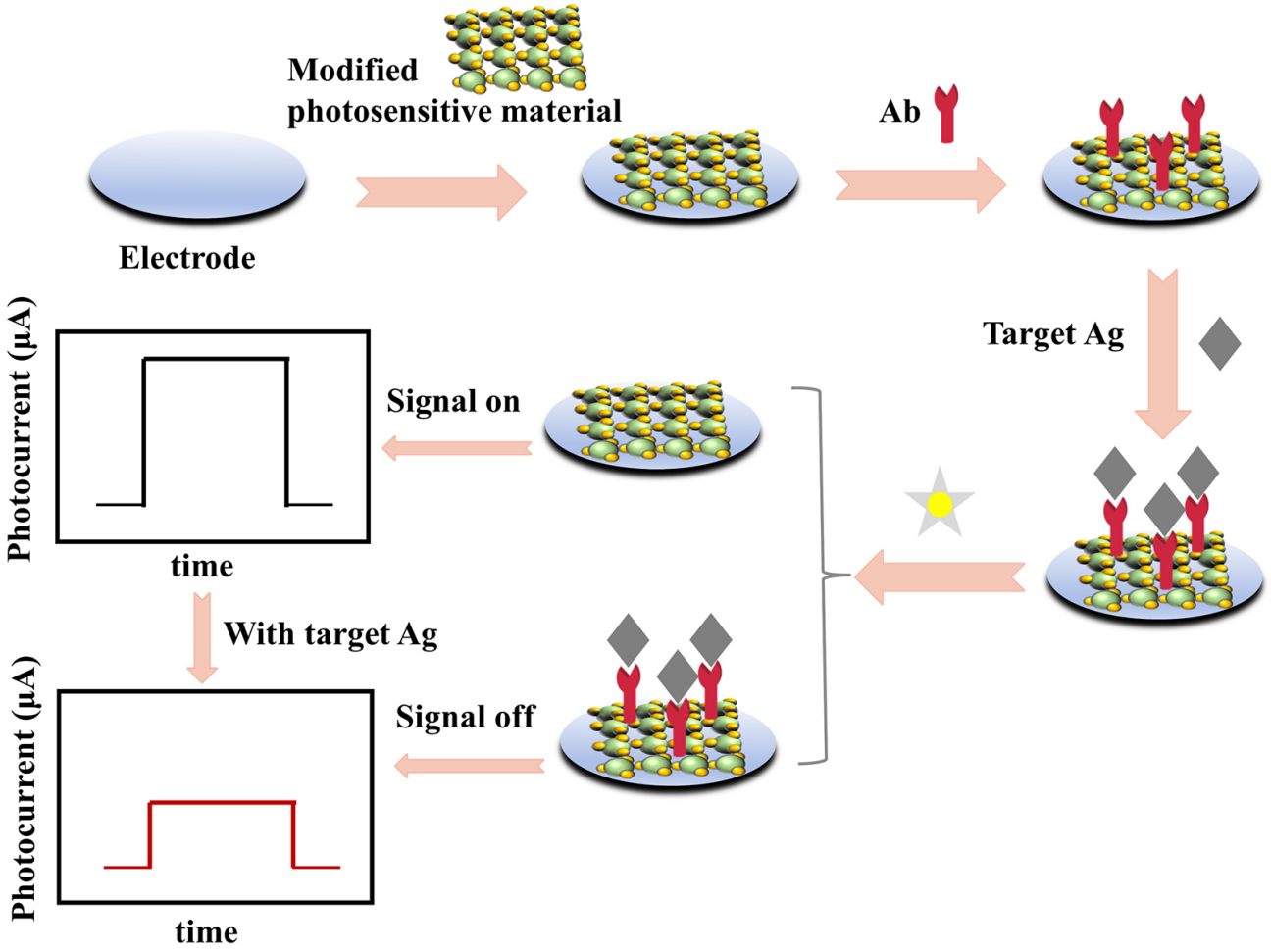

Fig. 6. Schematic diagram of modified photoelectrochemical (PEC) photoactive material modified electrode to enhance sensor sensitivity. The electrode is modified with a modified PEC photosensitive material to stimulate electron generation. After capturing the target antigen, the signal intensity decreases dramatically. This creates a "signal switch" sensing mode analysis platform. The key to the sensitive detection of biomolecules is the construction of highly efficient light-active materials. The figure was created using Microsoft Office 2016 (Microsoft, Redmond, WA, USA).

on the energy transfer mechanism, develop multifunctional nanomaterial emitters with unique electroluminescent properties, and explore efficient strategies for amplifying the ECL signal.

Photoelectrochemical Immunosensor

The photoelectrochemical (PEC) immunosensor irradiates the photoactive material on the electrode with a light source, and the electrons in the material are excited from the high-energy valence band to the low-energy conduction band and generate hole-electron pairs [82]. Under the action of potential, the photocurrent signal generated via the redox reaction of the electron donor in the solution can be used for the quantitative analysis of biomolecules [83]. In the process of PEC immunoassay, the amplification of biomolecule signals mainly lies in the PEC performance of the photoelectrically active materials, and the intensity of the signal changes upon binding with the target molecules. The more typical approaches are to construct PEC-active nanomaterials with excellent performance, modify the electrodes by means of modification, or introduce functional nanomaterials acting in the sensing mechanism according to the principle of the PEC reaction to provide the required power for that reaction. These design strategies will reduce the background signal interference to some extent and meet the immunoassay requirements [84]. In this section, the focus will be on the construction of high-performance photoelectrically active nanomaterials and the progress of research on nanomaterial strategies used in the PEC sensing mechanism and their clinical applications.

Construction of High-Performance PEC Photoactive Nanomaterials

Reviewing the development of PEC immunosensors, we can see that several types of photosensitive materials, such as organic compounds, polymers, transition metal

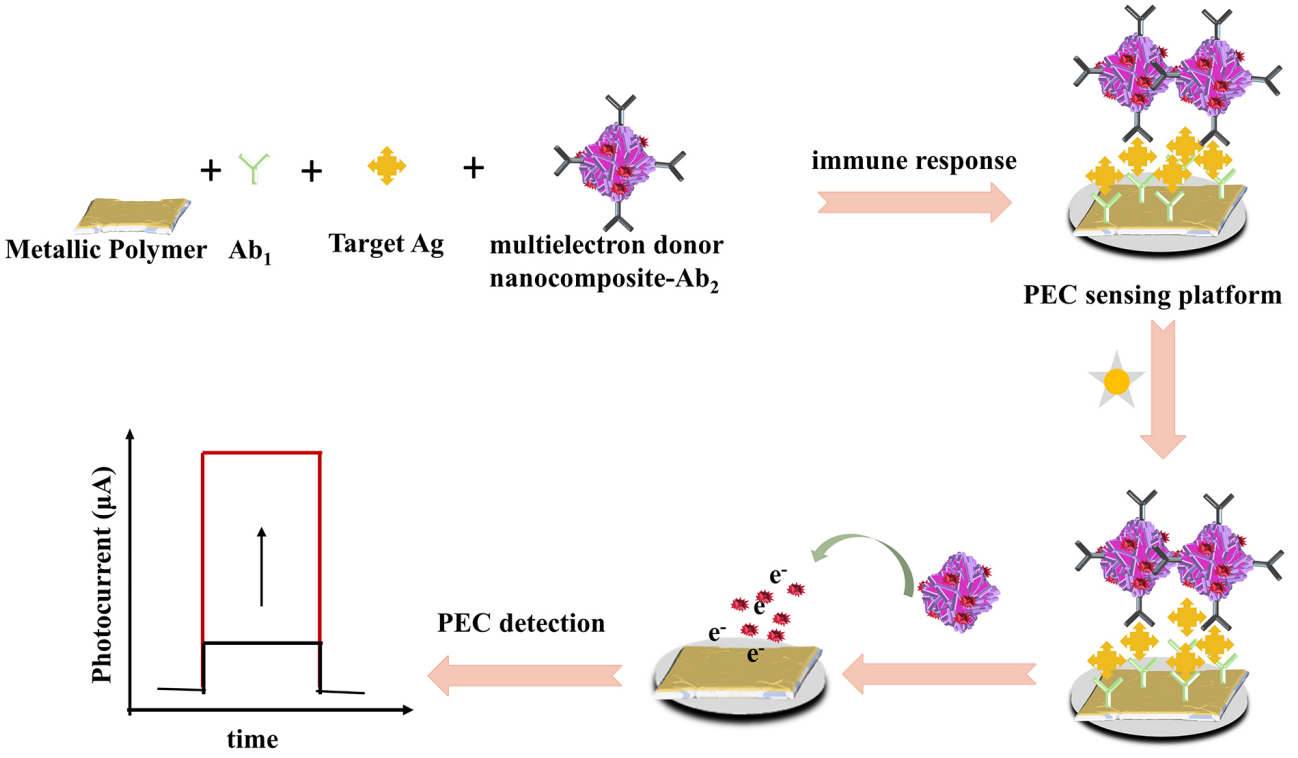

Fig. 7. Schematic representation of the role of the PEC sensing mechanism in organic multi-electron donor nanocomposites. The metal polymer modifies the electrode and fixes the primary antibody that recognizes the molecule, captures the target antigen and reacts immunologically with the secondary antibody labeled with the multi-electron donor nanocomposite material. Under light, the immunosensor platform releases the electron donor from the high-signal nanomaterial tags, preventing the recombination of electron-hole pairs and enhancing the PEC signal. The figure was created using Microsoft Office 2016 (Microsoft, Redmond, WA, USA).

complexes, and inorganic semiconductors, have been used in a large number of sensor structures. Semiconductor materials, in particular, have featured prominently in potential material applications due to their excellent photocatalytic activity and good biocompatibility [85]. However, most semiconductor materials have a wide and short photogenerated charge bandgap, which limits the detection sensitivity of PEC biomolecules. Therefore, here, we focus on analyzing the sensitivity effect brought about by the modified photosensitive material modifying the electrode. The basic design idea is shown in Fig. 6.

Compared to semiconductor materials, the elemental doping technique has become one of the most effective PEC signal amplification strategies due to its low cost, stable nature, and simple operation. For example, Fan *et al.* [86] designed nitrogen doped quantum dots as photocatalysts within the whole sensor, and the doped composite nanomaterials showed a strong anti-interference ability and high photoelectric activity, while the good chemical groups massively linked to capture molecules, and the PEC signals showed a strong change and a great increase in sensitivity after the addition of the target analyte, where N replaced lattice oxygen, resulted in a defective semiconductor material. This broadened the original light absorption range to include visible light and compensated for the loss of biomolecules caused by high-energy ultraviolet-visible light. Moreover, compared with non-metallic element doping, metallic element doping has a better optical response, that can better inhibit the recombination of electron-hole pairs and ultrasensitively enhance the response signal of the PEC immunoassay. Fan's team [87] designed an immunoassay device with Fe doped TiO_2 as a photosensitive material, where the Fe and TiO_2 intramolecular interactions changed the electronic state of the separate semiconductor material during signaling, enhancing the visible light range of the composite photosensitive material and its PEC response sensitivity. The signal intensity analysis of the captured tumor marker squamous cell carcinoma antigen (SCCA) resulted in a multifold enhancement upon the completion of the immune response.

The latest research method found, the use of ionic-based doping or polymer-modified composite nanomaterial technology, has been proposed as a promising method to enhance the optoelectronic performance of conventional semiconductors. This technology allows for the regulation of the energy band structure, resulting in improved energy transfer. Additionally, the conducting polymer's conjugated electron space system facilitates autonomous electron motion and enables the capture of a significant number of photoelectrons [88]. These various highly sensitive

sensing systems, constructed by combining modified nanomaterials with PEC immunosensing mechanisms, provide the basis for the application of PEC immunosensors in the detection of clinical low abundance tumor markers.

In order to further broaden the light absorption range and accelerate the separation and transfer of charge carriers, recently, new hot heterojunction nanocomposites have become popular due to the characteristics of photogenerated holes promoting chemical reactions. Examples include a semiconductor-semiconductor heterojunction, semiconductor-carbon heterojunction, metal-nanoparticle-quantum-dot heterojunction, and Z-type heterojunction. The two nanomaterials synthesized via a heterojunction make up for each other's defects, and the advantages cooperate to build a self-powered PEC sensing system [89] that is expected to be applied to more PEC immunoassay platform design schemes and offer a new approach to clinical disease diagnosis.

Nanomaterial Strategies Acting on Photoelectrochemical Sensing Mechanisms

In cases where the sensitivity design of modified photosensitive materials is limited, strategies to enhance the signaling effect of photoelectrochemical reactions based on the PEC-sensing mechanism have been explored in order to improve the situation. It has been found that different shapes of semiconductor photosensitive materials can absorb different wavelengths of light when they are combined with each other, and narrow bandgap quantum dots act as sensitizers to functionalize ordinary semiconductor materials, which are coupled to form a layered hierarchical co-sensitized structure. This layered structure synergistically produces a sensitizing effect, that can improve the light trapping ability of photosensitive materials [90]. Feng's team [91] was the first to employ the strategy of La-CdS sensitization of 3D $ZnIn_2S_4$ (synthesis by dissolution of zinc chloride, indium chloride and thioacetamide)/Au@ZnO to enhance the PEC immunoassay performance for the detection of NT-proBNP. In this case, when the target antigen was not added, the CdS semiconductors were matched with $ZnIn_2S_4$ energy levels to slow down charge complexation under light, while the lanthanum-doped structure further separated the charges, and the two synergistically provided a strong initial PEC signal. Afterwards, the PEC signal was linearly related to the target molecule as the concentration of the added target molecule increased, and the analytical effect was remarkable.

Another strategy for the maximum output signal sensitivity enhancement of PEC immunosensors is the development of signal-switching immunoassay platforms by exploiting the spatial site resistance of insulating biomolecules on electrodes. As a secondary antibody marker, multifunctional nanomaterials increase the steric hindrance effect on the electrode surface after the biological binding reaction [92], which leads to a significant decrease in the PEC signal, and the resulting photoelectric signal can be used to detect the concentration of immune biomolecules with high sensitivity. On the basis of this excellent analysis effect, combined with the high photoelectric conversion efficiency of the base photoactive nanomaterials, under the synergistic effect of the strong light trapping ability of the base material and the insulation of the bio-molecular porous material probe, the huge changes in the PEC photocurrent signal before and after the initial strong signal were observed [93].

In addition, the strong sensitivity-enhancing effect of enzymatic reactions cannot be ignored, and it has been introduced into sensor construction design in various ways. One of them is the use of multifunctional catalytic materials as labeled antibodies to catalyze the generation of a large number of electron donors from H_2O_2 *in situ* to inhibit the recombination of electron-hole pairs, achieving an enhanced degree of photocurrent signal response, which is of great significance for the monitoring of reactive oxygen species, which are closely related to clinical diseases [94]. With the same mechanism of action, the ions released through chemical reactions *in situ* can regulate electron transfer on the surface of the photosensitive material, maximizing the electron transfer efficiency and meeting detection requirements [95]. As shown in Fig. 7, Chen's group [96] used conjugated compounds, graphene, and dopamine to prepare an organic multi-electron donor nanocomposite. The innovation of this research work is that the organic multi-electron donor composite nanomaterial does not need to add an additional electron donor during the immunosensing process, because its own structure can be sensitized under light to generate the electron donor required for the reaction, and this low background signal design strategy achieves a detection limit of 3.6 fg mL^{-1} for the tumor marker CEA. It also provides a good direction for future bioanalytical studies into organic photovoltaic materials.

Electrochemical, electrochemiluminescent, and photoelectrochemical immunosensing technologies offer many benefits for the early diagnosis of cancer and the analysis of human health, as shown in Table 2 (Ref. [32,46,49,54,65,71,78,81,86,96–105]), and these innovative and efficient nanomaterials strategies can facilitate the design of immunosensors with different analytical performance profiles, which, in turn, lead to advances in clinical immunoassays.

Future Prospectives

In the future, it will be necessary to explore more economical, low-toxicity, easy-to-design nanomaterial strategies with excellent immunoassay performance. These nanomaterial technologies will not only enhance our understanding of nanomaterial immunosensing mechanisms, but also lay the foundation for exploring the develop-

Table 2. Analytical performance characteristics of typical immunosensors constructed with nanomaterial strategies.

Types of immunosensing analysis	Nanomaterials design strategy	Target Analyte	Detection Limit	Linear range	Ref.
Electrochemical	Large specific surface area material molybdenum disulfide loaded noble metal nanoparticles as substrate material for modified electrodes	CA242	3.43×10^{-5} U·mL^{-1}	1×10^{-4}–1×10^{2} U·mL^{-1}	[32]
	Preparation of WL Pt nanomaterials with excellent electrocatalytic properties for label-free detection platforms	AFP	0.028 pg mL^{-1}	0.0001–100 ng mL^{-1}	[97]
	Preparation of high-performance immunosensor chips using dendritic gold dendrites (AuDdrites)	25-hydroxyvitamin D3	0.03 ng mL^{-1}	0.1–900 ng mL^{-1}	[46]
	Host and object recognition technique mediated by gold nanoparticles	CEA	0.034 ng mL^{-1}	0.11 ng mL^{-1}–80 ng mL^{-1}	[49]
	Multimolecular simultaneous detection of metal ion functionalized composite sensing interface	CgA, CgB	5.3(CgA) and 2.1(CgB) fg mL^{-1}	0.1 pg mL^{-1}–100 ng mL^{-1}	[98]
	TSA technique mediated electrocatalytic precipitation of electroactive substance Fc-Tyr	GPC3	0.087 ng mL^{-1}	0.1 ng mL^{-1}–1 μg mL^{-1}	[54]
Electrochemical luminescence	Controllable ECL detection platform of Luminol functionalized nanoparticles based on bipolar electrode	CEA	2.51 ng mL^{-1}	5–300 ng mL^{-1}	[99]
	Co-reaction promoter composite nanomaterial CuS/HPC modified electrode to promote the conversion of $K_2S_2O_8$ into active intermediate substances for luminescent systems	CA242	0.015 mU mL^{-1}	0.1 mU mL^{-1}–100 U mL^{-1}	[65]
	Self-enhanced ECL luminescence system constructed by combining co-reactants and luminescent materials into one by chemical bonding	caspase-3	0.017 pg mL^{-1}	0.05–200 pg mL^{-1}	[71]
	The nanochannel array enhances the signal by growing nanocatalysts *in situ*	CEA	0.03 pg mL^{-1}	0.1 pg mL^{-1}–1000 ng mL^{-1}	[100]
	ECL-RET sensor platform based on double potential emitters	NSE	0.041 pg mL^{-1}	0.0001–200 ng mL^{-1}	[101]
	Unlabeled potential-resolved ECL detection platform based on PRMCECL luminescent group	cTnI	5.01 fg mL^{-1}	0.01–1000 pg mL^{-1}	[78]
	Carbon nanohorns with photothermal properties were used as thermal conversion units to increase the electrode surface temperature and further enhance the ECL signal	HE4	3.3×10^{-6}ng mL^{-1}	1.0×10^{-5}–10 ng mL^{-1}	[81]
Photoelectrochemical	PEC immunoassay platform based on novel element doped photosensitive materials	cTnI	0.3 pg mL^{-1}	0.001–100 ng mL^{-1}	[86]
	ITO electrode was modified with heterojunction BiOI/Bi_2S_3 material	CYFRA21-1	1.72 pg mL^{-1}	0.001–100 ng mL^{-1}	[102]
	Peptide-based PEC sensing platform based on sensitized structure electrode	PSA	0.0015 ng mL^{-1}	0.005–20 ng mL^{-1}	[103]
	Double signal quenching effect	CA199	0.0004 U mL^{-1}	0.001–50 U mL^{-1}	[104]
	DA-ZnTCPP-g-C_3N_4 with multiple electron donors to build a “signal on” sensor	CEA	3.6 fg·mL^{-1}	10 fg·mL^{-1}–1 mg·mL^{-1}	[96]
	Multi-nanocomposite energy level matching	NSE	0.07 pg mL^{-1}	0.1 pg mL^{-1}–50 ng mL^{-1}	[105]

AFP, alpha-fetoprotein; TSA, tyramine signal amplification; HPC, 2D high porous carbon; ITO, indium tin oxide; NSE, neuron-specific enolase; cTnI, cardiac troponin I; HE4, human epididymis protein 4; g-C_3N_4, graphitic carbon nitride; CYFRA21-1, Cytokeratin-19-fragment; PSA, prostate-specific antigen; CA199, carbohydrate antigen 19-9; GPC3, Glypican3.

ment of new sensor diagnostic technologies. Nanomaterial-mediated immunosensors link science and technology with life in today's era of rapid development of smart internet technology and promote the continuous innovation and progress of bio-diagnostic technology. Based on these innovative new strategies, the construction of reliable, specific, and stable electrochemical, electrochemiluminescent, and photoelectrochemical immunosensors that can be applied in clinical departments to assist in diagnosis is an important direction for the development of tumor marker detection. Through analysis, the nanomaterials strategy has broad applicability and can help related researchers to understand the bioanalytical applications of immunosensors, and this comprehensive evaluation framework provides strong support and guidance for building top-quality immunosensing platforms.

The combination of the design versatility of nanomaterials and the convenience and specificity of immunosensors will contribute to the booming development of many fields in the future, such as instant clinical detection, home diagnostics, and industry and agriculture, etc. In order to make full use of the signal amplification potential of nanomaterials, it is of great significance to explore the role of the relationship between the two at a deeper level.

Conclusions

In summary, it is clear that nanomaterial-mediated electrochemical, electrochemiluminescent, and photoelectrochemical immunosensors offer a number of advantages over strategies such as enzyme cascade amplification and nucleic acid amplification in the detection of tumor markers. These advantages include significantly enhanced sensitivity, suitable cost-effectiveness, and ease of manipulation. Several nanomaterial strategies have been reported to construct immunosensors. Among them, for EC immunosensors, the use of highly conductive noble metals synergistically interacting with large specific surface area carbon-based materials to modify the electrodes or the use of modified high-performance composites as signal labels to promote the interfacial reaction remain the classical analytical technique compared to the other two immunosensor types; ECL immunosensors for both single- and dual-signals, the traditional luminescent moieties with functionalized nanomaterials synergistic effects from the nanomaterials; and the innovation of novel emitters, all meet the requirements of immunoassay to a certain extent, but it cannot be ignored that there is an unavoidable non-specific selection of non-target substances; in contrast, in PEC immunosensors, the clever design of functionalized nanomaterials provides a low limit of detection and minimal background interference during the sensing process, which makes them useful for the detection of low-abundance tumor biomarkers and small molecular biomarkers in the inflammatory or tumor microenvironment. These nanomaterial strategies are reflected in the impact on the performance of immunosensors in terms of sensitivity, stability, and specificity. It is emphasized that we need to fully understand the sensing mechanisms of various immunosensors and synergize these nanomaterial strategies with the principle mechanisms of the immunosensors for sensitivity amplification.

However, the detection and analysis performance of immunosensors, although constantly being optimized, still face many challenges in clinical applications. Currently, most immunosensor applications are still dominated by laboratory research and have not yet fully entered clinical diagnostic applications, which require further research for improvement. The development of new immunosensors has only been useful in research institutes and schools, and has not really entered the market to be fully utilized; the reproducibility of the sensors still needs to be improved, which is a major aspect affecting the analytical performance of the sensors.

Moreover, as discussed in this paper, the nanomaterial design strategy has some advantages in some reported signal amplification techniques for immunosensors, but there is a wide variety of nanomaterials, and the rational design of nanomaterials and their successful application to real samples is an issue that needs to be seriously considered.

Abbreviations

AFP, alpha-fetoprotein; Ag, antigen; Ab_2, the secondary antibody; EC, electrochemical; ECL, electrochemiluminescence; PEC, photoelectrochemical; Au NPs , gold nanoparticles; GO, graphene; $Ru(bpy)_3^{2+}$, Tris(bipyridine)ruthenium(II) ion; QDs, quantum dots; g-C_3N_4, graphitic carbon nitride; ECL-RET, electrochemiluminescence resonance energy transfer; H_2O_2, hydrogen peroxide; MOFs, metal-organic frameworks.

Author Contributions

YPX—conceptualization, data curation and writing original draft (lead); XLL, XYL—data curation, writing-original draft (supporting); YH—conceptualization, supervision and funding acquisition. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgment

Not applicable.

Funding

This work was supported in part by the National Nature Science Foundation of China (No. 82072340); the Guangxi Science and Technology Major Program (No. AA24011005); the Scientific and Technological Innovation Major Base of Guangxi (No.2022-36-Z05).

Conflict of Interest

The authors declare no conflict of interest.

References

[1] de Visser KE, Joyce JA. The evolving tumor microenvironment: From cancer initiation to metastatic outgrowth. Cancer Cell. 2023; 41: 374–403.

[2] Thun MJ, DeLancey JO, Center MM, Jemal A, Ward EM. The global burden of cancer: priorities for prevention. Carcinogenesis. 2010; 31: 100–110.

[3] Li M, Jiang F, Xue L, Peng C, Shi Z, Zhang Z, *et al*. Recent Progress in Biosensors for Detection of Tumor Biomarkers. Molecules (Basel, Switzerland). 2022; 27: 7327.

[4] Chen L, Chen Y, Feng YL, Zhu Y, Wang LQ, Hu S, *et al*. Tumor circulome in the liquid biopsies for digestive tract cancer diagnosis and prognosis. World Journal of Clinical Cases. 2020; 8: 2066–2080.

[5] Japp NC, Souchek JJ, Sasson AR, Hollingsworth MA, Batra SK, Junker WM. Tumor Biomarker In-Solution Quantification, Standard Production, and Multiplex Detection. Journal of Immunology Research. 2021; 2021: 9942605.

[6] Laraib U, Sargazi S, Rahdar A, Khatami M, Pandey S. Nanotechnology-based approaches for effective detection of tumor markers: A comprehensive state-of-the-art review. International Journal of Biological Macromolecules. 2022; 195: 356–383.

[7] Sharma KK, Moshin M, Mittal P, Ali Z, Fatma N, Upadhyay P, *et al*. Diagnosis of the Initial Stage of Hepatocellular Carcinoma: A Review. Current Pharmaceutical Design. 2024; 30: 1708–1724.

[8] Mummareddy S, Pradhan S, Narasimhan AK, Natarajan A. On Demand Biosensors for Early Diagnosis of Cancer and Immune Checkpoints Blockade Therapy Monitoring from Liquid Biopsy. Biosensors. 2021; 11: 500.

[9] Azzouzi S, Ben Ali M, Bellagambi F, Elaissari A, Jaffrezic-Renault N, Errachid A, *et al*. Spatially hierarchical nanoarchitecture for real time detection of Interleukin-8 cancer biomarker. Talanta. 2022; 246: 123436.

[10] Shen J, Situ B, Du X, Wang Z, Hu R, Li B, *et al*. Aggregation-Induced Emission Luminogen-Based Dual-Mode Enzyme-Linked Immunosorbent Assay for Ultrasensitive Detection of Cancer Biomarkers in a Broad Concentration Range. ACS Sensors. 2022; 7: 766–774.

[11] Dai Y, Chiu LY, Chen Y, Qin S, Wu X, Liu CC. Neutral Charged Immunosensor Platform for Protein-based Biomarker Analysis with Enhanced Sensitivity. ACS Sensors. 2019; 4: 161–169.

[12] Olejnik B, Kozioł A, Brzozowska E, Ferens-Sieczkowska M. Application of selected biosensor techniques in clinical diagnostics. Expert Review of Molecular Diagnostics. 2021; 21: 925–937.

[13] Mohamad Nor N, Ridhuan NS, Abdul Razak K. Progress of Enzymatic and Non-Enzymatic Electrochemical Glucose Biosensor Based on Nanomaterial-Modified Electrode. Biosensors. 2022; 12: 1136.

[14] Zheng S, Li M, Li H, Li C, Li P, Qian L, *et al*. Sandwich-type electrochemical immunosensor for carcinoembryonic antigen detection based on the cooperation of a gold-vertical graphene electrode and gold@silica-methylene blue. Journal of Materials Chemistry. B. 2020; 8: 298–307.

[15] Qin J, Li J, Zeng H, Tang J, Tang D. Recent advances in metal-organic framework-based photoelectrochemical and electrochemiluminescence biosensors. The Analyst. 2023; 148: 2200–2213.

[16] Mollarasouli F, Kurbanoglu S, Ozkan SA. The Role of Electrochemical Immunosensors in Clinical Analysis. Biosensors. 2019; 9: 86.

[17] Ramya PR, Halder S, Nagamani K, Singh Chouhan R, Gandhi S. Disposable graphene-oxide screen-printed electrode integrated with portable device for detection of SARS-CoV-2 in clinical samples. Bioelectrochemistry. 2024; 158: 108722.

[18] Wang C, Liu S, Ju H. Electrochemiluminescence nanoemitters for immunoassay of protein biomarkers. Bioelectrochemistry (Amsterdam, Netherlands). 2023; 149: 108281.

[19] Fiorani A, Valenti G, Iurlo M, Marcaccio M, Paolucci F. Electrogenerated chemiluminescence: A molecular electrochemistry point of view. Current Opinion in Electrochemistry. 2018; 8: 31–38.

[20] Yang L, Zhang S, Liu X, Tang Y, Zhou Y, Wong DKY. Detection signal amplification strategies at nanomaterial-based photoelectrochemical biosensors. Journal of Materials Chemistry. B. 2020; 8: 7880–7893.

[21] Chen L, Yang G, Qu F. Aptamer-based sensors for fluid biopsies of protein disease markers. Talanta. 2024; 276: 126246.

[22] Mo T, Liu X, Luo Y, Zhong L, Zhang Z, Li T, *et al*. Aptamer-based biosensors and application in tumor theranostics. Cancer Science. 2022; 113: 7–16.

[23] Li M, Singh R, Wang Y, Marques C, Zhang B, Kumar S. Advances in Novel Nanomaterial-Based Optical Fiber Biosensors-A Review. Biosensors. 2022; 12: 843.

[24] Fritea L, Banica F, Costea TO, Moldovan L, Dobjanschi L, Muresan M, *et al*. Metal Nanoparticles and Carbon-Based Nanomaterials for Improved Performances of Electrochemical (Bio)Sensors with Biomedical Applications. Materials (Basel, Switzerland). 2021; 14: 6319.

[25] Qiu Z, Tang D. Nanostructure-based photoelectrochemical sensing platforms for biomedical applications. Journal of Materials Chemistry. B. 2020; 8: 2541–2561.

[26] Xiao Y, Zhang T, Zhang H. Recent advances in the peptide-based biosensor designs. Colloids and Surfaces. B, Biointerfaces. 2023; 231: 113559.

[27] Rogers JK, Taylor ND, Church GM. Biosensor-based engineering of biosynthetic pathways. Current Opinion in Biotechnology. 2016; 42: 84–91.

[28] Kim J, Park M. Recent Progress in Electrochemical Immunosensors. Biosensors. 2021; 11: 360.

[29] Robinson C, Juska VB, O'Riordan A. Surface chemistry applications and development of immunosensors using electrochemical impedance spectroscopy: A comprehensive review. Environmental Research. 2023; 237: 116877.

[30] Azharuddin M, Zhu GH, Das D, Ozgur E, Uzun L, Turner APF, *et al*. A repertoire of biomedical applications of noble metal nanoparticles. Chemical Communications (Cambridge, England). 2019; 55: 6964–6996.

[31] Cho IH, Lee J, Kim J, Kang MS, Paik JK, Ku S, *et al*. Current Technologies of Electrochemical Immunosensors: Perspective on Signal Amplification. Sensors (Basel, Switzerland). 2018; 18: 207.

[32] Cao L, Lu S, Guo C, Chen W, Gao Y, Ye D, *et al*. A novel electrochemical immunosensor based on PdAgPt/MoS_2 for the ultrasensitive detection of CA 242. Frontiers in Bioengineering and Biotechnology. 2022; 10: 986355.

[33] Kurbanoglu S, Ozkan SA. Electrochemical carbon based nanosensors: A promising tool in pharmaceutical and biomedical analysis. Journal of Pharmaceutical and Biomedical Analysis. 2018; 147: 439–457.

[34] Silva RM, da Silva AD, Camargo JR, de Castro BS, Meireles LM, Silva PS, *et al*. Carbon Nanomaterials-Based Screen-Printed Electrodes for Sensing Applications. Biosensors. 2023; 13: 453.

[35] Ranjan P, Abubakar Sadique M, Yadav S, Khan R. An Electrochemical Immunosensor Based on Gold-Graphene Oxide Nanocomposites with Ionic Liquid for Detecting the Breast Cancer CD44 Biomarker. ACS Applied Materials & Interfaces. 2022; 14: 20802–20812.

[36] Akbari Jonous Z, Shayeh JS, Yazdian F, Yadegari A, Hashemi M, Omidi M. An electrochemical biosensor for prostate cancer biomarker detection using graphene oxide-gold nanostructures. Engineering in Life Sciences. 2019; 19: 206–216.

[37] Xie Y, Tu X, Ma X, Fang Q, Lu L, Yu Y, *et al*. High-performance voltammetric sensor for dichlorophenol based on β-cyclodextrin functionalized boron-doped graphene composite aerogels. Nanotechnology. 2019; 30: 185502.

[38] Durmaz H. A graphene-metal hybrid biosensor for SEIRA spectroscopy applications. Optics Communications. 2024; 550.

[39] Chen Z, Li H, Chen Z, Xuan X, Zhou B, Li M. Two-channel electrochemical immunosensor based on one-step-synthesized AuPt-boron-doped graphene electrode for CA153 detection. Biosensors & Bioelectronics. 2023; 222: 114974.

[40] Derakhshi M, Daemi S, Shahini P, Habibzadeh A, Mostafavi E, Ashkarran AA. Two-Dimensional Nanomaterials beyond Graphene for Biomedical Applications. Journal of Functional Biomaterials. 2022; 13: 27.

[41] Dutta K, De S, Das B, Bera S, Guria B, Ali MS, *et al*. Development of an Efficient Immunosensing Platform by Exploring Single-Walled Carbon Nanohorns (SWCNHs) and Nitrogen Doped Graphene Quantum Dot (N-GQD) Nanocomposite for Early Detection of Cancer Biomarker. ACS Biomaterials Science & Engineering. 2021; 7: 5541–5554.

[42] Safari M, Moghaddam A, Salehi Moghaddam A, Absalan M, Kruppke B, Ruckdäschel H, *et al*. Carbon-based biosensors from graphene family to carbon dots: A viewpoint in cancer detection. Talanta. 2023; 258: 124399.

[43] Cao L, Zhang W, Lu S, Guo C, Wang P, Zhang D, *et al*. A Label-Free Electrochemical Immunosensor for CEA Detection on a Novel Signal Amplification Platform of Cu_2S/Pd/CuO Nanocomposites. Frontiers in Bioengineering and Biotechnology. 2021; 9: 767717.

[44] Ramanavicius S, Samukaite-Bubniene U, Ratautaite V, Bechelany M, Ramanavicius A. Electrochemical molecularly imprinted polymer based sensors for pharmaceutical and biomedical applications (review). Journal of Pharmaceutical and Biomedical Analysis. 2022; 215: 114739.

[45] Tang C, Zhang JX, Chen DN, He JW, Wang AJ, Feng JJ. Ultrasensitive label-free electrochemical immunosensor of NT-proBNP biomarker based on branched AuPd nanocrystals/N-doped honeycombed porous carbon. Bioelectrochemistry (Amsterdam, Netherlands). 2022; 148: 108225.

[46] Martins TS, Bott-Neto JL, Machado SAS, Oliveira ON, Jr. Label-Free Electrochemical Immunosensor Made with Tree-like Gold Dendrites for Monitoring 25-Hydroxyvitamin D3 Metabolite. ACS Applied Materials & Interfaces. 2022; 14: 31455–31462.

[47] Jiang M, Liao J, Liu C, Liu J, Chen P, Zhou J, *et al*. Metal-organic frameworks/metal nanoparticles as smart nanosensing interfaces for electrochemical sensors applications: a mini-review. Frontiers in Bioengineering and Biotechnology. 2023; 11: 1251713.

[48] Hasanzadeh M, Mohammadzadeh A, Jafari M, Habibi B. Ultrasensitive immunoassay of glycoprotein 125 (CA 125) in untreated human plasma samples using poly (CTAB-chitosan) doped with silver nanoparticles. International Journal of Biological Macromolecules. 2018; 120: 2048–2064.

[49] Liang H, Luo Y, Li Y, Song Y, Wang L. An Immunosensor Using Electroactive COF as Signal Probe for Electrochemical Detection of Carcinoembryonic Antigen. Analytical Chemistry. 2022; 94: 5352–5358.

[50] Jing P, Zhao C, Yin ZZ, Yang B, Li J, Cai W, *et al*. An electrochemical chiral sensor based on competitive host-guest interaction for the discrimination of electroinactive amino acids. Analyst. 2022; 147: 5068–5074.

[51] Shao Y, Zhou H, Wu Q, Xiong Y, Wang J, Ding Y. Recent advances in enzyme-enhanced immunosensors. Biotechnology Advances. 2021; 53: 107867.

[52] Chi Z, Wang Q, Gu J. Recent advances in colorimetric sensors based on nanozymes with peroxidase-like activity. The Analyst. 2023; 148: 487–506.

[53] Fang X, Liu J, Wang J, Zhao H, Ren H, Li Z. Dual signal amplification strategy of Au nanopaticles/ZnO nanorods hybridized reduced graphene nanosheet and multienzyme functionalized Au@ZnO composites for ultrasensitive electrochemical detection of tumor biomarker. Biosensors & Bioelectronics. 2017; 97: 218–225.

[54] Lu W, Xie X, Lan X, Wu P, Peng H, He J, *et al*. An electrochemical immunosensor for the detection of Glypican-3 based on enzymatic ferrocene-tyramine deposition reaction. Biosensors & Bioelectronics. 2023; 225: 115081.

[55] Bölükbaşi ÖS, Yola BB, Karaman C, Atar N, Yola ML. Electrochemical α-fetoprotein immunosensor based on Fe_3O_4NPs@covalent organic framework decorated gold nanoparticles and magnetic nanoparticles including SiO_2@TiO_2. Mikrochimica Acta. 2022; 189: 242.

[56] Thapa K, Liu W, Wang R. Nucleic acid-based electrochemical biosensor: Recent advances in probe immobilization and signal amplification strategies. Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology. 2022; 14: e1765.

[57] Sano T, Smith CL, Cantor CR. Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates. Science (New York, N.Y.). 1992; 258: 120–122.

[58] Xu L, Duan J, Chen J, Ding S, Cheng W. Recent advances in rolling circle amplification-based biosensing strategies-A review. Analytica Chimica Acta. 2021; 1148: 238187.

[59] Kirschbaum SEK, Baeumner AJ. A review of electrochemiluminescence (ECL) in and for microfluidic analytical devices. Analytical and Bioanalytical Chemistry. 2015; 407: 3911–3926.

[60] Mwanza C, Ding SN. Newly Developed Electrochemiluminescence Based on Bipolar Electrochemistry for Multiplex Biosensing Applications: A Consolidated Review. Biosensors. 2023; 13: 666.

[61] Sabzehmeidani MM, Kazemzad M. Quantum dots based sensitive nanosensors for detection of antibiotics in natural products: A review. The Science of the Total Environment. 2022; 810: 151997.

[62] Nesakumar N, Srinivasan S, Alwarappan S. Graphene quantum dots: synthesis, properties, and applications to the development of optical and electrochemical sensors for chemical sensing. Mikrochimica Acta. 2022; 189: 258.

[63] Das A, Bej S, Pandit NR, Banerjee P, Biswas B. Recent advancements of metal–organic frameworks in sensing platforms: relevance in the welfare of the environment and the medical sciences with regard to cancer and SARS-CoV-2. Journal of Materials Chemistry A. 2023; 11: 6090–6128.

[64] Han S, Zhao Y, Zhang Z, Xu G. Recent Advances in Electrochemiluminescence and Chemiluminescence of Metal Nan-

oclusters. Molecules. 2020; 25: 5208.
[65] Shen C, Li Y, Li Y, Wang S, Li Y, Tang F, *et al*. A double reaction system induced electrochemiluminescence enhancement based on SnS_2 QDs@MIL-101 for ultrasensitive detection of CA242. Talanta. 2022; 247: 123575.
[66] Zhang JX, Lv CL, Tang C, Jiang LY, Wang AJ, Feng JJ. Ultrasensitive sandwich-typed electrochemical immunoassay for detection of squamous cell carcinoma antigen based on highly branched PtCo nanocrystals and dendritic mesoporous SiO_2@AuPt nanoparticles. Mikrochimica Acta. 2022; 189: 416.
[67] Li J, Lai W, Jiang M, Li P, Wang M, Ma C, *et al*. Enhancing Ru(bpy)32+@TMU-13 electrochemiluminescence for ultrasensitive detection of AFP by a signal amplification strategy based on flower-like Au NPs/CoFe LDO/MoS2 NFs as double coreaction accelerators. Sensors and Actuators B: Chemical. 2023; 393.
[68] Hu L, Shi T, Chen J, Cui Q, Yu H, Wu D, *et al*. Dual-quenching electrochemiluminescence resonance energy transfer system from CoPd nanoparticles enhanced porous g-C_3N_4 to FeMOFs-sCuO for neuron-specific enolase immunosensing. Biosensors & Bioelectronics. 2023; 226: 115132.
[69] Jin L, Liu W, Xiao Z, Yang H, Yu H, Dong C, *et al*. Recent Advances in Electrochemiluminescence Biosensors for Mycotoxin Assay. Biosensors. 2023; 13: 653.
[70] Wu J, Wu Y, Bian H, Peng Z, Liu Y, Yin Y, *et al*. Fabrication of a ratiometric electrochemiluminescence biosensor using single self-enhanced nanoluminophores for the detection of spermine. Talanta. 2023; 253: 123880.
[71] Luo W, Chu H, Wu X, Ma P, Wu Q, Song D. Disposable biosensor based on novel ternary Ru-PEI@PCN-333(Al) self-enhanced electrochemiluminescence system for on-site determination of caspase-3 activity. Talanta. 2022; 239: 123083.
[72] Cao Y, Zhou JL, Ma Y, Zhou Y, Zhu JJ. Recent progress of metal nanoclusters in electrochemiluminescence. Dalton Transactions (Cambridge, England: 2003). 2022; 51: 8927–8937.
[73] Azimzadeh M, Khashayar P, Amereh M, Tasnim N, Hoorfar M, Akbari M. Microfluidic-Based Oxygen (O_2) Sensors for On-Chip Monitoring of Cell, Tissue and Organ Metabolism. Biosensors. 2021; 12: 6.
[74] Liu X, Bai L, Cao X, Wu F, Yin T, Lu W. Rapid determination of SARS-CoV-2 nucleocapsid proteins based on 2D/2D MXene/P-BiOCl/Ru(bpy)$_3^{2+}$ heterojunction composites to enhance electrochemiluminescence performance. Analytica Chimica Acta. 2022; 1234: 340522.
[75] Zhang HR, Xu JJ, Chen HY. Electrochemiluminescence ratiometry: a new approach to DNA biosensing. Analytical Chemistry. 2013; 85: 5321–5325.
[76] Feng QM, Shen YZ, Li MX, Zhang ZL, Zhao W, Xu JJ, *et al*. Dual-Wavelength Electrochemiluminescence Ratiometry Based on Resonance Energy Transfer between Au Nanoparticles Functionalized g-C3N4 Nanosheet and Ru(bpy)3(2+) for microRNA Detection. Analytical Chemistry. 2016; 88: 937–944.
[77] Luo S, Zeng Z, Zeng G, Liu Z, Xiao R, Chen M, *et al*. Metal Organic Frameworks as Robust Host of Palladium Nanoparticles in Heterogeneous Catalysis: Synthesis, Application, and Prospect. ACS Applied Materials & Interfaces. 2019; 11: 32579–32598.
[78] Du D, Shu J, Guo M, Haghighatbin MA, Yang D, Bian Z, *et al*. Potential-Resolved Differential Electrochemiluminescence Immunosensor for Cardiac Troponin I Based on MOF-5-Wrapped CdS Quantum Dot Nanoluminophores. Analytical Chemistry. 2020; 92: 14113–14121.
[79] Fang D, Huang Y, Zhang S, Dai H, Hong Z, Lin Y. Versatile NiCo2O4 nanosheets hybrids-based label-free immunosensor for thyroglobulin using photothermal amplification. Electrochimica Acta. 2020; 337.
[80] Ni J, Zhang H, Chen Y, Luo F, Wang J, Guo L, *et al*. DNAzyme-based Y-shaped label-free electrochemiluminescent biosensor for lead using electrically heated indium-tin-oxide electrode for in situ temperature control. Sensors and Actuators B: Chemical. 2019; 289: 78–84.
[81] Fang D, Zhang S, Dai H, Lin Y. An ultrasensitive ratiometric electrochemiluminescence immunosensor combining photothermal amplification for ovarian cancer marker detection. Biosensors & Bioelectronics. 2019; 146: 111768.
[82] Del Barrio M, Luna-López G, Pita M. Enhancement of Biosensors by Implementing Photoelectrochemical Processes. Sensors (Basel, Switzerland). 2020; 20: 3281.
[83] Kozinetz A, Tsymbalyuk O, Litvinenko S. Application of sensor structures based on a photoelectric transducer to determine the activity of aspartate and alanine aminotransferases in blood plasma. Biomedical Physics & Engineering Express. 2023; 9.
[84] Zhang B, An Z, Li M, Guo LH. Synthesis, functionalization and photoelectrochemical immunosensing application of WO3-based semiconductor materials. TrAC Trends in Analytical Chemistry. 2023; 165.
[85] Di YM, Liu JY, Li MH, Zhang SQ, You MH, Lin MJ. Donor-Acceptor Hybrid Heterostructures: An Emerging Class of Photoactive Materials with Inorganic and Organic Semiconductive Components. Small. 2022; 18: e2201159.
[86] Fan D, Liu X, Shao X, Zhang Y, Zhang N, Wang X, *et al*. A cardiac troponin I photoelectrochemical immunosensor: nitrogen-doped carbon quantum dots-bismuth oxyiodide-flower-like SnO_2. Mikrochimica Acta. 2020; 187: 332.
[87] Fan D, Wu D, Cui J, Chen Y, Ma H, Liu Y, *et al*. An ultrasensitive label-free immunosensor based on CdS sensitized Fe-TiO2 with high visible-light photoelectrochemical activity. Biosensors & Bioelectronics. 2015; 74: 843–848.
[88] Wang C, Tang Y, Zhang B, Zhong Z, Zhao F, Zeng B. Sensitive photoelectrochemical immunosensor for carcinoembryonic antigen detection based on copolymer of thiophene and thiophene-3-acetic acid modified phosphate-doped Bi_2WO_6. Analytica Chimica Acta. 2023; 1262: 341243.
[89] Dos Santos WS, Carmo ÉJ, Mendez-González Y, Nascimento LL, Patrocínio AOT, Guo R, *et al*. Innovative multifunctional hybrid photoelectrode design based on a ternary heterojunction with super-enhanced efficiency for artificial photosynthesis. Scientific Reports. 2020; 10: 10669.
[90] Monteiro TO, Neto AGDS, de Menezes AS, Damos FS, Luz RDCS, Fatibello-Filho O. Photoelectrochemical Determination of Cardiac Troponin I as a Biomarker of Myocardial Infarction Using a Bi_2S_3 Film Electrodeposited on a $BiVO_4$-Coated Fluorine-Doped Tin Oxide Electrode. Biosensors. 2023; 13: 379.
[91] Feng J, Li F, Li X, Wang Y, Fan D, Du B, *et al*. Label-free photoelectrochemical immunosensor for NT-proBNP detection based on La-CdS/3D $ZnIn_2S_4$/Au@ZnO sensitization structure. Biosensors & Bioelectronics. 2018; 117: 773–780.
[92] Guo A, Pei F, Feng S, Hu W, Zhang P, Xia M, *et al*. A photoelectrochemical immunosensor based on magnetic all-solid-state Z-scheme heterojunction for SARS-CoV-2 nucleocapsid protein detection. Sensors and Actuators B: Chemical. 2023; 374: 132800.
[93] Zhang N, Li Y, Zhao G, Feng J, Li Y, Wang Y, *et al*. Ultrasensitive photoelectrochemical sensing platform for detection of neuron specific enolase based on inhibition effect of CoSnO3 nanobox toward SnO2/Mn0.05Cd0.95S composites. Talanta. 2023; 253.
[94] Wang GL, Shu JX, Dong YM, Wu XM, Li ZJ. An ultrasensitive and universal photoelectrochemical immunoassay based on enzyme mimetics enhanced signal amplification. Biosensors & Bioelectronics. 2015; 66: 283–289.
[95] Bott Neto JL, Martins TS, A. S. Machado S, Oliveira ON.

Enhanced photocatalysis on graphitic carbon nitride sensitized with gold nanoparticles for photoelectrochemical immunosensors. Applied Surface Science. 2022; 606.

[96] Chen M, Guo J, Mo F, Yu W, Fu Y. Highly Sensitive Photoelectrochemical Immunosensor Based on Organic Multielectron Donor Nanocomposite as Signal Probe. Analytical Chemistry. 2022; 94: 17039–17045.

[97] Li SS, Tan YY, Zhang Y, Liu M, Liu A. A simple electrochemical immunosensor based on worm-like platinum for highly sensitive determination of alpha-fetoprotein. Bioelectrochemistry (Amsterdam, Netherlands). 2021; 140: 107804.

[98] Liu X, Li Y, He L, Feng Y, Tan H, Chen X, *et al*. Simultaneous detection of multiple neuroendocrine tumor markers in patient serum with an ultrasensitive and antifouling electrochemical immunosensor. Biosensors & Bioelectronics. 2021; 194: 113603.

[99] Mohammadniaei M, Zhang M, Qin X, Wang W, Pia L, Gürbüz H, *et al*. A hand-held electrochemiluminescence biosensor for detection of carcinoembryonic antigen. Talanta. 2024; 266: 125087.

[100] Zhang H, Zhang C, Qu H, Xi F. Immunosensor with Enhanced Electrochemiluminescence Signal Using Platinum Nanoparticles Confined within Nanochannels for Highly Sensitive Detection of Carcinoembryonic Antigen. Molecules (Basel, Switzerland). 2023; 28: 6559.

[101] Dong X, Du Y, Zhao G, Cao W, Fan D, Kuang X, *et al*. Dual-signal electrochemiluminescence immunosensor for Neuron-specific enolase detection based on “dual-potential” emitter $Ru(bpy)_3^{2+}$ functionalized zinc-based metal-organic frameworks. Biosensors & Bioelectronics. 2021; 192: 113505.

[102] Zhang S, Wang C, Wu T, Fan D, Hu L, Wang H, *et al*. A sandwiched photoelectrochemical biosensing platform for detecting Cytokeratin-19 fragments based on Ag_2S-sensitized $BiOI/Bi_2S_3$ heterostructure amplified by sulfur and nitrogen co-doped carbon quantum dots. Biosensors & Bioelectronics. 2022; 196: 113703.

[103] Zhao J, Wang S, Zhang S, Zhao P, Wang J, Yan M, *et al*. Peptide cleavage-mediated photoelectrochemical signal on-off via CuS electronic extinguisher for PSA detection. Biosensors & Bioelectronics. 2020; 150: 111958.

[104] Zhang Y, Chen J, Wang H, Cui Q, Fan D, Zhang Y, *et al*. Novel Photoelectrochemical Biosensing Platform Based on a Double Type II $CdLa_2S_4/SnIn_4S_8/Sb_2S_3$ Ternary Heterojunction as Photoactive Materials and $NiCo_2O_4$ Nanospheres as a Photoquencher for CA19-9 Detection. Analytical Chemistry. 2022; 94: 15915–15923.

[105] Zhang Y, Wu T, Liu D, Xu R, Ma H, Wei Q, *et al*. Photoelectrochemical immunosensor for the sensitive detection of neuron-specific enolase based on the effect of Z-scheme $WO_3/NiCo_2O_4$ nanoarrays p-n heterojunction. Biosensors & Bioelectronics. 2022; 213: 114452.