Review

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Electrochemical Sensor Based on Nanomaterials and Its Application in the Detection of Alpha Fetoprotein

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Hepatocellular carcinoma development and many other tumors are closely related to alpha-fetoprotein (AFP), its determination can be used as a positive test for tumors. It is mainly used clinically as a serum marker to diagnose and monitor the efficacy of primary hepatocellular carcinoma. Therefore, a variety of biosensors have been developed to detect AFP. Electrochemical sensors integrate a variety of detection methods. They have inherent advantages over other types of sensors, they are fast, portable, simple, and highly sensitive. Some meaningful electrochemical biosensors work with nanomaterials acting as signal amplification elements or as signal amplification catalysts. This review introduced the field of biosensors and discuss about the use of nanomaterials in electrochemical sensing, specificity electrochemical biosensing of AFP. The study ends with a discussion about the prospects for nanomaterial-based signal amplification and future research directions.

Keywords: electrochemical sensor; signal amplification; nanomaterials; alpha-fetoprotein; nanoparticles; nanotubes; graphene

Introduction

Cancer is a major global public health problem and the second leading cause of death in the US. According to the American Cancer Society, new cancer cases and cancer related death increased in 2022 compared to 2021, in US. Fear of cancer is a common phenomenon at this stage [1,2]. Fortunately, some cancers, such as colon, liver, lung, cervical, and breast cancers, can be detected via screening, which can help delay or even stop cancer progression [3]. Therefore, timely and accurate screening for earlystage cancers is particularly important to reduce cancer mortality [4]. The most common screening methods available include pathological tissue biopsy, tumor imaging and tumor markers. The first two methods are very limited to detect early tumors. Some methods are expensive and cause pain and harm to patients, while tumor markers are less invasive, less costly, easier to interpret and more common in clinical screening. However, the low concentration of tumor markers in early stages may result in a certain rate of missed diagnoses. Thus, it is particularly important to design a detection strategy with high specificity and sensitivity for early-stage tumor markers [5].

Alpha-fetoprotein (AFP) is a serum protein that characterizes fetal development. It is the most reliable and widely used tumor marker to diagnose yolk sac tumors and hepatocellular carcinoma [6]. AFP overexpression has been widely reported to be associated with the development and prognosis of many tumors, such as lung cancer [7], pancreatic neuroendocrine tumors [8] and cervical cancer [9]. AFP threshold is 20 ng/mL [10]. To date, many methods have been used to measure AFP, including mass spectrometry [11], fluorescence [12], chemiluminescence assay [13], enzyme-linked immunosorbent assay [14], radioimmunoassay [15], and electrochemistry. Among these strategies, electrochemical immunoassays have been widely developed and are the prefer technique to quantitatively detect AFP, due to their inherent advantages, including low detection lines, low-cost ease of handling, and small amounts of analytes. Electrochemical immunosensors have excellent properties of nanomaterials for signal amplification and show significant advantages in terms of selectivity and sensitivity [16].

Nanomaterials are materials that have at least one dimension in three dimensions (1–100 nm) or are composed of them as basic units, corresponding to a scale of about 10–1000 atoms packed tightly together. Nanomaterials are increasingly used in the field of biological analysis [17–19]. So far, some nanomaterials with different characteristics have been used for electrochemical sensing, including metal nanoparticles [20], carbon nanomaterials [21], quantum dots [22], polymers [23], and semiconductor materials [24]. They are often used to enhance sensing signals and to detect ultra-low concentrations of small molecules and biomarkers. In addition, nanomaterials are of great interest to researchers due to their large specific surface area, abundant binding sites, moldable nanoscale shapes, high stability properties, and biocompatibility [25–28]. Nanomaterials are often used as electroactive substances [29], nanocarriers [30], and nano-enzymes [31], and they have different functional roles for signal amplification in electrochemical sensors. Fig. 1 describes AFP abnormality and its use for diagnosis. In this review, the recent research progress and development of electrochemical biosensors to detect AFP based on nanomaterials is discussed. In addition, the construction principle of biosensors based on nanomaterials and signal amplification application of nanomaterials to determine AFP are also described.

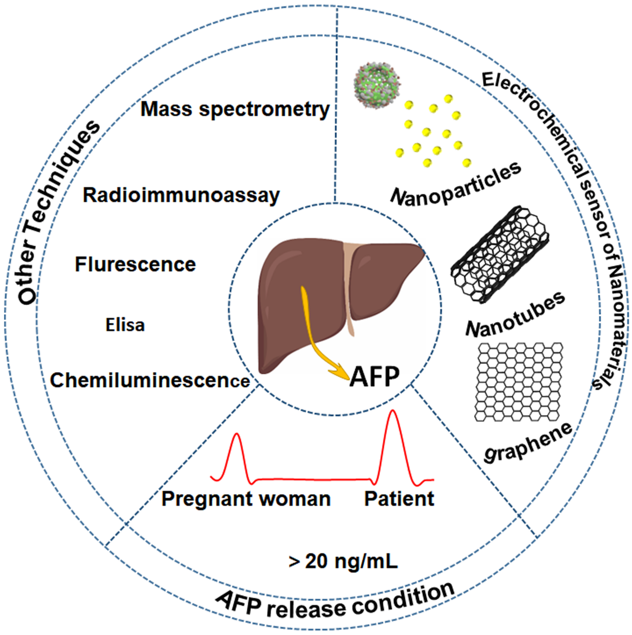

Fig. 1. Describes AFP abnormality and its use for diagnosis.

Electrochemical Signal Amplification Based on Nanomaterials

Electrochemical sensing is established by the change of the electrical output signal, resulting from the chemical reaction between the identifying element on the electrode and the target analyte. It can be used for qualitative detection and quantitative analysis of target molecules by generating electrical signals related to target analyte concentration [32]. In nanomaterial related electrochemical sensors, nanomaterials are fixed on the electrode surface working as recognition elements. The change in capacitance caused by the analyte binding or the change in current or potential resulting from the redox reaction on the electrode surface is then evaluated by the specific binding of the nanomaterial to the target. Depending on the type of response signal, cyclic voltammetry, resistive resistance a different ampere method can be used [33–35]. This section discusses the different functional applications of nanomaterials in electrochemistry based on the principles of simple construction, mainly in terms of non-enzymatic signal amplification and enzymatic catalytic signal amplification.

Non-Enzymatic Signal Amplification

In most cases, the electrochemical signal amplification is mainly fixed on the electrode or electrode sheet by chemical or physical methods for surface modification. It can be used as a switching device to fix antigen, antibody or target proteins to promote electron transmission, reduce background current, and increase signal-to-noise ratio, enhancing electrochemical detection sensitivity and realizing signal amplification. Wang *et al.* [36] used gold nanoparticles (Au NPs) modified with poly (glycidyl methacrylate) to increase electrodes' surface area and introduce enough antibody sites to increase antibodies accessibility to analytes for tumor markers signal amplification. Gu *et al.* [37] designed Au NPs to increase the electrical conductivity of the sensing surface while carrying immobilized secondary anti-cancer embryonic antigens and electroactive ferrocene derivatives to detect carcinoembryonic antigen (CEA) in human serum. On the other hand, electrode sensing interface with excellent conductivity and biocompatibility is constructed using nanomaterials as the substrate to enhance the response signal. Scala-Benuzzi *et al.* [38] doped electroreduced graphene on the electrode surface to increase conductivity and reduce electrode resistance and electrodeposited porous gold structures. This increases the surface area and immobilize more antibodies to capture most of the target protein for signal amplification.

In most studies, the two aspects are often combined for double signal amplification. In other words, nanomaterials are used as the base to enhance the response signal and surface modification. Luo *et al.* [39] used reduced graphene oxide-gold nanoparticles (rGO-Au NPs) as substrate material to modify the electrode to increase sensing interface specific surface area and conductivity, and modified the surface via single-walled carbon nanotubes (SWCNTs) @ graphene quantum dots (GQDs) composite material. Double signal amplification results are generated, as shown in Fig. 2A.

Enzyme Signal Amplification

Enzyme-catalyzed amplification is an early application for electrochemical sensors. Enzymes are highly efficient, specific, selective, and tunable, but biological enzymes have significant drawbacks, with milder conditions of action, susceptibility to environmental influences, and they require timeconsuming and expensive purification processes, limiting their application in electrochemical sensors. Zhou *et al.* [40] produced horseradish peroxidase (HRP) complexes with platinum nanoparticles (Pt NPs) and nucleophosmintargeted aptamers (CP) to catalyze tyramine sig-

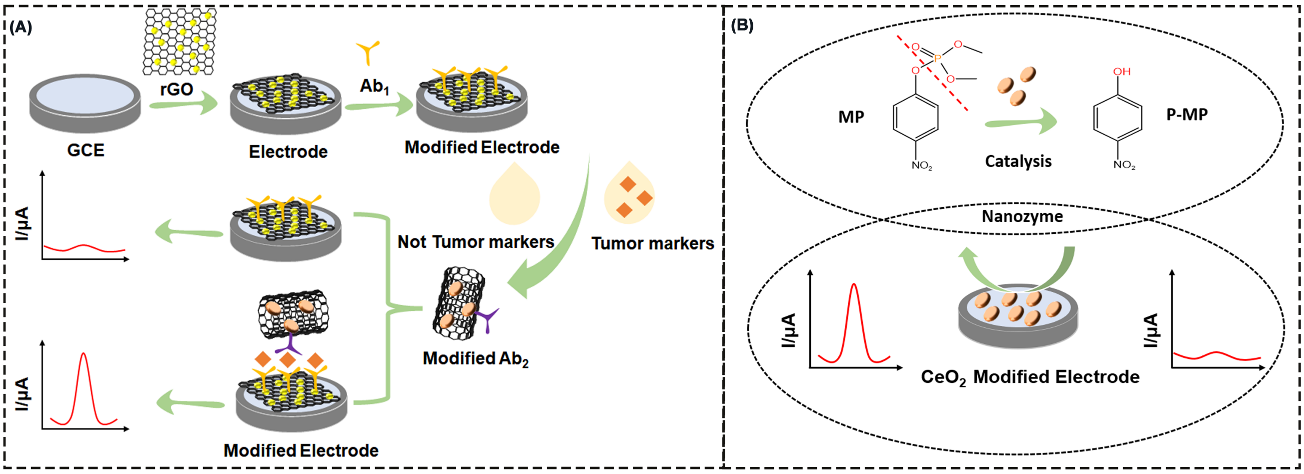

Fig. 2. Schematic diagram of signal amplification structure based on nanomaterials. (A) Non-enzyme signal amplification. (B) Nanozyme signal amplification.

nal amplification for circulating tumor cells sensitive detection. But Pt NPs@HRP@CP complexes synthesis requires overnight reactions at 4 °C and constant attention to temperature and acidity during sensor preparation.

In order to avoid natural enzymes defects, different types of artificial simulated enzymes have been synthesized and applied successively, such as cyclodextrin, polymeric polymer, porphyrins, and biomacromolecules. Alizadeh *et al.* [41] used a DNAzyme simulation with horseradish peroxide activity, composed of G-quadruplex DNA and heme molecules to catalyze hydrogen peroxide reduction with the help of methylene blue. This process results in signal amplification to determine hepatitis B virus surface antigen. Although they can mimic enzyme active center, the catalytic activity is still limited compared to that of natural enzymes. With the progress of nanotechnology, nanoenzymes with unique physical and chemical properties of nano-materials as well as enzymatic activity have been synthesized and applied. Different from natural enzymes and artificial simulated enzymes, nano-enzymes not only have a simple preparation process, low cost, can be modified and storage easily, are reusable, and are acid, alkali, and heat resistance, but also are capable of effective catalytic activity of nano-enzymes regulation by changing nano-enzymes size, morphology, surface and composition. Thanks to those advantages, nanozyme are widely used in electrochemical sensors. Sun *et al.* [42] reported a bifunctional cerium oxide (CeO_2) nanozyme (Fig. 2B). CeO_2 with phosphatase mimetic activity degrades methyl-paraoxon to p-nitrophenol. The CeO_2-modified nanozyme electrode continued to detect p-nitrophenol. Nanometer enzymes with bifunctional properties are used to eliminate biological enzymes shortcomings and amplify the electrochemical detection signal.

AFP Detection Based on Nanomaterials

Nanoparticles

Compared to conventional electrochemical biosensors, new electrochemical sensors based on nanomaterials have superior performance. Nanoparticles are widely used in electrochemical sensors due to their excellent properties, such as simple preparation, easy adsorption, and good biocompatibility. Electrochemical sensors have received increased attention in the electrochemistry field [43,44]. This is not only because they are of great scientific value but also because they establish a link between matter and atoms or molecules. When nanoparticles are used for electrochemical sensors construction, significant signal amplification can be achieved.

Shen *et al.* [45] used a method based on polyamide amide tree macromolecules (PAMAM) encapsulated Au NPs (Au-PAMAM) to increase electrode surface area and accelerate electron dynamics to detect AFP. Au-PAMAM was the first covalently immobilized on a gold electrode, followed by electrochemical redox marker ferrocene ionic liquid sequential covalent immobilization and the AFP. Monoclonal antibody, after immobilized AFP immunorecognition to its antibody, the peak ferrocene current was reduced due to the blocked electron transfer reaction on the electrode surface, and a low 0.02 ng/mL was achieved by differential pulse voltammetry to detect AFP detection line. Compared to Elisa results, it is clear that the use of combined electrochemistry and Au NPs can significantly improve assay sensitivity.

To further amplify detection signal and improve sensitivity and stability, Gao *et al.* [46] used a triple signal amplification composite for AFP ultrasensitive detection. Due to their good biocompatibility and specific electronic configuration, Au nanoparticles are widely used as attachment sites and support materials. In this study, molybdenum disulfide @ cuprous oxide-gold nanoparticles were used to indirectly

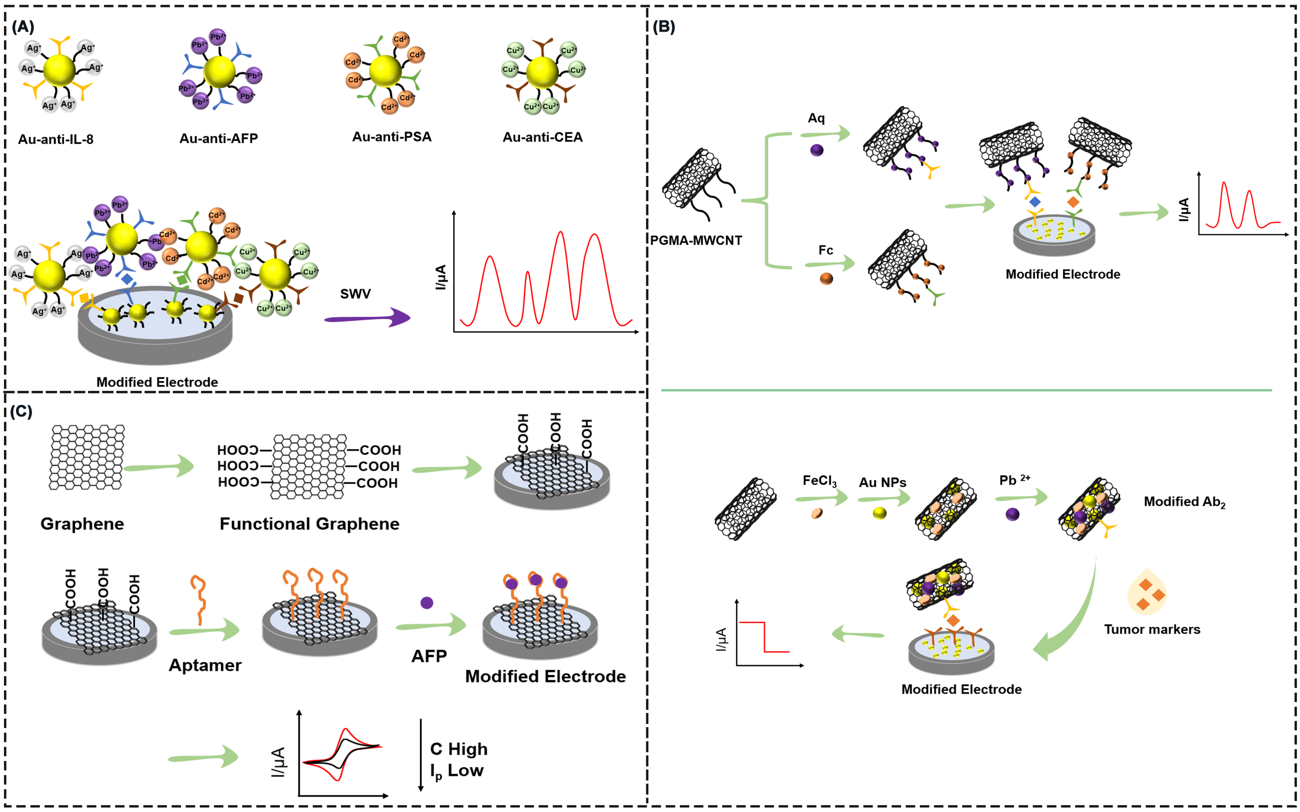

Fig. 3. Sensing diagram of signal amplification for tumor marker detection based on different nanomaterials. (A) Nanoparticles. (B) Functional nanotubes. (C) Functionalized graphene with aptamer.

detect AFP by catalyzing the current response to hydrogen peroxide, resulting in a linear range of high sensitivity (0.1 pg/mL–50 ng/mL) and a 0.037 pg/mL low detection line. In addition, a human serum assay was performed, and satisfactory recoveries were obtained. In order to obtain a simple and sensitive electrochemical sensor, Zhou *et al.* [47] synthesized gold nanoparticles-dextran-reduced graphene oxide nanocomposite for AFP detection. Ferrocyanide was used as an electrochemical probe. Ferricyanide peak redox current intensity has a linear relationship with AFP concentration. The detection limit (LOD) is 0.05 pg/mL and the detection range is 0.01–20 ng/mL. Its specificity, selectivity and stability are also acceptable and can have good results in serum specimen clinical application.

Significantly, many researchers have reported that nanoparticles can be used as different markers to detect different tumor markers. Liu *et al.* [48] reported a novel electrochemical immunosensor for AFP and CEA simultaneous detection. The sensor is based on a one-step synthesized redox active polymer/Au nanocomposite material, and poly (vinyl ferrocene-2-aminothiophenol)/Au nanocomposite and poly (o-phenylenediamine)/Au nanocomposite are used as medium to contact two different antibodies. Differential pulse voltammetry is used to detect two kinds of electroactive substances. The results showed that electrochemical immunosensor can simultaneously detect two kinds of tumor markers. Further, Putnin *et al.* [49] proposed a multifunctional material for simultaneously detecting four tumor markers. The sensor uses polyethyleneimine-coated gold nanoparticles adsorbed on a screen-printed carbon electrode for modification and labeling. The electrodes are also loaded with electrically active metal ions cadmium (II), lead (II), copper (II) and silver (I) and are associated to secondary antibodies, which give different voltammetry signals at –0.80, –0.55, –0.20 and +0.05 V, respectively (Fig. 3A). Four corresponding trapping antibodies (Ab_1) were then coupled to an electrode. After forming a sandwich structure, by combining the analyte with labeled Au NPs, the corresponding electrochemical signal response was recorded, with AFP linear range of 0.25–10 ng/mL. The investigator's strategy provides opportunities for immunosensors development for tumor biomarkers multianalyte assay, early diagnosis, and multi-target detection in clinical applications. In addition, Sun *et al.* [50] proposed two unlabeled and sandwich-type electrochemical immunosensors based on bimetallic gold-platinum nanoparticles vertical graphene for AFP sensitive detection, which plays a crucial role in early diagnosis of primary liver cancer. Under optimal experimental conditions, sandwich sensor sensitivity is higher than that of the unlabeled sensor, its linear width reaches 1 fg/mL–100 ng/mL, and the LOD is 0.7 fg/mL. Based on the excellent performance of immunosensor to detect AFP in human serum and its good stability, researchers believe that it will be used in AFP clinical monitoring and other tumor markers. Most researchers prefer to use Au NPs to build biosensors, not only because of the simplicity of preparation and good biocompatibility but also because Au NPs have good electron transport and stable chemical bonding. In addition to Au NPs, other metals or metal oxidation nanoparticles also show good biocompatibility and can bind with enzyme or antibody functional groups without damaging their biological activity [51], which is widely used in electrochemical sensors.

Nanotubes

Nanotube has enriched nanotechnology field and has been widely used in various fields. Nanotubes are nanoscale materials with a tubular shape. Some of them are carbon nanotubes, inorganic nanotubes, peptide nanotubes and nucleic acid nanotubes [52]. Among them, carbon nanotubes are hollow cylinders rolled up by graphite layers, which are mainly manifested in two forms; SWCNTs and multi-walled carbon nanotubes (MWCNTs) [53].

In recent years, carbon nanotubes have been widely used in electrochemical sensors due to their excellent physical and chemical properties [54–56]. Compared with other types of nanotubes, carbon nanotubes (CNTs) are not easily affected by the environment, and have strong adsorption characteristics, so they are often used as carriers of other nanomaterials [57]. In addition, carbon nanotubes have good flexibility, solid compressive resistance and superior charge transfer ability. Hence, as electrode materials for electrochemical biosensors, carbon nanotubes have unique advantages [58].

In recent years, many researchers have changed the diversified research from the general characterization of CNTs types to the study of more unique and exciting electrical analysis characteristics, which has caught the attention of modified carbon nanotubes in the field of electrochemistry. Walled carbon nanotubes surface functionalization will provide additional capacity for enhanced chemical modification (Fig. 3B). Thus, Li *et al.* [59] prepared goldfunctionalized magnetic multi-walled carbon nanotubes (Au@MWCNTs-Fe_3O_4) for better adsorption of lead ions and secondary antibodies. Au@MWCNTs-Fe_3O_4 has a synergistic effect after lead ions adsorption and has a good catalytic effect on hydrogen peroxide. As a signal marker, an ultra-sensitive sandwich AFP electrochemical immunosensor was developed, and the linear range of ultra-sensitive (10 fg/mL–100 ng/mL) was obtained. The detection line was as low as 3.33-fg/mL. Compared with Peng *et al.* [60] polystyrene composite nanotube (0.0088 ng/mL), the sensor developed by Li *et al.* [59] has a tremendously excellent linear range and detection line. Researchers confirm that this electrochemical sandwich immunosensor has high sensitivity, good selectivity and stability to quantitatively detect AFP. Moreover, it has excellent potential for clinical and diagnostic applications.

Carbon nanotubes application as bioanalytical probes has been widely reported. To make CNTs easily functionalized by biomolecules, a variety of polymers have been used as sensing composites. Wang *et al.* [61] reported an electrochemical immune sensor based on surface-to-initiated poly (glycidyl methacrylate) and MWCNTs, which can detect CEA and AFP simultaneously. MWCNTs have good electrical conductivity, low resistance and high specific surface area, making it attractive for electrochemistry. However, MWCNTs may not be able to hold more signal molecules, which may limit detection signals expansion. Therefore, special polymers were used to functionalize MWCNTs, which significantly reduced non-specific adsorption and adsorbed more signal molecules. The results showed that AFP linear range was 100 fg/mL–100 ng/mL, and the detection line was as low as 32.8 fg/mL, while CEA linear range was as low as 163 fg/mL–163 ng/mL, and the detection line was as low as 56.1 fg/mL, indicating high sensitivity and selectivity. It is expected to be used for multiple tumor markers simultaneous detection.

While carbon nanotubes have many engineering applications, they are not safe for use in health care, while other types of nanotubes are favored because of their non-toxic water treatment technology [62]. Titanium dioxide (TiO_2) nanomaterials have received special attention due to their inherent advantages, including large surface area, high stability, simple synthesis, environmentally friendly, non-toxic and adjustable size, among other properties. Consequently, Huo *et al.* [63] reported an electrochemical immune sensor for TiO_2 nanotubes. A ternary TiO_2 nanotube (TNT) complex was designed, with antibodies and horseradish peroxidase functionalized TNT as electrode scaffolders and tracer markers, respectively. TNTs were coated with polyaniline (PANI) by chemical oxidation polymerization, and Au NPs were deposited on TNT-PANI surface by conventional chemical reduction. Compared with other carbon nano-sensors, the immune sensor has a wide linear range (0.01–350 ng/mL), and the LOD is 1.5 pg/mL. Researchers believe that this immune sensor component can be extended to other marker recognition systems and provide good stability, reproducibility and accuracy, making it potentially suitable for biological analysis and clinical diagnosis.

Graphene

Graphene and its related materials have been widely studied and applied in many fields in the past nineteen years. Graphene and related materials are currently being mass-produced and used in biomedical technologies, defining new standards for drug delivery and biosensing [64]. Graphene is a two-dimensional nanomaterial with a hexagonal honeycomb lattice composed of carbon atoms with sp2 hybridized orbitals. It is characterized by a large specific surface area, strong electron transfer ability, good electrical conductivity, good biocompatibility and high carrier mobility, which provides excellent conditions for preparing high-performance sensors [65–68].

However, many studies have shown that intrinsic graphene has weak physical adsorption to most gas molecules [69,70]. Thus, graphene and vacancy defect graphene concepts have been proposed to solve this problem. The introduction of defect or doping atoms can effectively enhance the charge transfer between graphene and gas molecules, improve the adsorption capacity of some molecules, and enhance the sensitivity and selectivity of graphene-based sensors. rGO contains residual oxygen and other heteroatoms, as well as structural defects, and it is often used in the electrochemistry field. It has excellent conductivity and a large specific surface area, making it is suitable for loading nanoparticles or metal ions to maximize electron transfer. Qi *et al.* [71] prepared palladium-graphene nanocomplex with a simple method, synthesized Pdhybrid rGO with carbon monoxide, and reduced carbon monoxide adsorbed on palladium surface. As a carrier, sensitive labelling-free immune sensor was constructed with the lowest detection line reached 5 pg/mL.

Although rGO is an attractive material similar to the original graphene, the presence of oxygen-containing groups not only makes graphene oxide (GO) chemically stable but also provides the active site for surface modification and a large specific surface area. GO can be obtained by adding different types of oxygencontaining functional groups, such as hydrocarbons, carboxyl groups, and carbonyl groups, on graphene surface [72]. Thus, GO may be an ideal sensor material. Wei *et al.* [73] bind antibodies by graphene amino functionalization. At the same time, octahedral palladium particles carrier, the electrode surface is modified to realize electrode signal amplification. The LOD of this method is 0.033 pg/mL. Yang *et al.* [74] combined carboxy-functionalized graphene with aptamers to form a simple sensor (Fig. 3C). Experimental results showed that this method can obtain a wide linear range of 0.01–100 ng/mL and a low detection line of 3 pg/mL. Hu *et al.* [75] reported a highly sensitive electrochemical immunochip for AFP and CEA, using 3D graphene aerogel integrated bedside detection (POCT). Various POCT platforms have been developed and applied to detect tumor markers, among which POCT based on electrochemistry has high specificity and sensitivity, which has attracted much attention. Here, the author overcomes the challenge of detection platform miniaturization, not only retaining microfluidic technology advantages but detecting tumor markers in a timely and sensitive way. This has great application potential in cancer early detection and diagnosis. Rapid diagnosis of tumor biomarker at bedside is expected to receive significant attention and research in the future.

Conclusions and Perspectives

In conclusion, over the past decade, AFP has been critical for cancer treatment prognosis, early diagnosis of liver cancer, and auxiliary diagnosis of other tumors. In this review, signal amplification strategy induced by nanomaterials and the construction and detection method of AFP electrochemical sensing interface is introduced in detail. New nanomaterials have become a research hotspot in biomarkers real-time detection for early liver cancer due to their advantages in electrochemical application. In addition, AFP electrochemical sensors performance is improving thanks to the development of functionalization and diversification of nanomaterials. However, there are still fundamental issues to overcome. Electrochemical stability and repeatability need to be improved. Antibodies are widely used in electrochemical sensing. However, there are still many problems in recognizing multiple target sites, and it is not easy to identify the slight differences between large molecular targets such as proteins. In order to overcome these shortcomings, research can be performed to introduce a variety of new nanomaterials with better binding sites and bonding ability. In addition, some specific recognition sites are presented, including aptamer introduction on the surface of nanomaterials for recognition, which can realize the detection of various proteins, cells, viruses or antibiotics. This review also mentioned that a simple combination of aptamers with graphene can result in a wide linear range and sensitivity. Therefore, the combination of aptamer functionalized novel nanomaterials and electrochemical signal amplification can accurately detect tumor markers in future studies. Although some challenges and problems remain, the combination of electrochemical nanomaterials and signal amplification shows clear and exciting applications. With POCT development, microchips and nanomaterials variety with superior performance further increases electrochemical sensors analytical performance. It is expected to expand their immediate detection in clinical practice. In the foreseeable future, electrochemical sensors based on nanomaterials will be widely used in clinical settings.

Abbreviations

AFP, alpha-fetoprotein; Au NPs, gold nanoparticles; CEA, carcinoembryonic antigen; rGO, reduced graphene oxide; LOD, limit of detection; HRP, horseradish peroxidase; Pt NPs, platinum nanoparticles; CP, nucleophosmin targeted aptamers; CeO_2, cerium oxide; PAMAM, polyamide amide tree macromolecules; Ab_1, antibodies; SWCNTs, single-walled carbon nanotubes; MWCNTs, multi-walled carbon nanotubes; Fe_3O_4, ferroferric oxide; GQDs, graphene quantum dots; CNTs, carbon nanotubes; TiO_2, titanium dioxide; TNT, titanium dioxide nanotube; PANI, polyaniline; GO, graphene oxide; POCT, bedside detection.

Availability of Data and Materials

Materials will be made available on request.

Author Contributions

RPH—conceptualization, data curation and writing-original draft (lead); ZKZ, LG, DFF, ZBQ, XJS—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.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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