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Review

# An Overview on Electrochemical Approaches for the Detection of Alpha-Fetoprotein

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**Abstract**

Cancer biomarkers have a remarkable role for not only early-stage detection of cancer but also in the monitoring therapy efficacy. Alpha-fetoprotein (AFP) is one of the biomarkers which is highly expressed in hepatocellular carcinoma (HCC) patients. Therefore, a high level of AFP can be considered for early diagnosis of HCC. Electrochemical assays can be used as the miniaturized biosensors for real-time detection of the analytes which are mostly categorized into three groups for AFP detection including immunosensors, aptasensors, and molecularly imprinted polymer-based sensors. All of these techniques provide beneficial detection features of fast and cost-effective compared with other analytical methods. This review describes the most recent electrochemical platforms for AFP detection to provide a perspective for future studies.

## Introduction

Early diagnosis is the fundamental concept in cancer effective and safe treatment which can be accessed by monitoring of cancer biomarkers.[1,2] Although other diagnostic techniques like magnetic resonance imaging and computerized tomography (CT) have been applied in this way, it is impossible to repeat them in high-risk patients who need to be checked regularly.[3,4] Cancer biomarkers have a remarkable role not only in an early-stage detection of cancer but also in the monitoring of therapy efficacy.[5,6] Alpha-fetoprotein (AFP) is one of the most important cancer biomarkers with 70 kDa molecular weight which is highly expressed in hepatocellular carcinoma (HCC) patients. In 75% of HCC patients, the AFP amount elevates up to 500 ng/mL, while the normal level should be less than 25 ng/mL.[7,8] Therefore, a high level of AFP can be considered for early diagnosis of HCC.

Previously, some analytical assays were suggested for AFP determination including fluorescence,[9] chemiluminescence,[10] radioimmunoassay (RIA),[11] enzyme-linked immunosorbent assay (ELISA),[12] etc. These methods are high-cost and need sophisticated instruments which consequently lead to long-run and time-consuming operations. However, electrochemical-based approaches can be used as miniaturized platforms for fast and point-of-care detection of the analytes.[13-21] In this way, electrochemical biosensors of AFP, based on the affinity agents, are categorized into three main groups; *i*) immunosensors, in which the electrode surface is modified by antibody immobilization. Through this complexation, the electron transfer is prevented that creating the redox activity in an electrolyte (through insulating protein layer formation),[22] *ii*) aptasensors, in which the aptamers are the recognition structure to detect an analyte. Aptamers are short single-stranded DNA or RNA sequences and have high affinity to the different biological targets (from small-sized nucleic acids to large-sized proteins and even whole cells) based on their tertiary structure,[23] and *iii*) molecularly imprinted polymer (MIP)-based sensors, consist on target structure modification on the polymer network and incorporation on the surface of the electrode.[22]

Here, the most recent electrochemical sensing platforms for AFP detection have been summarized and their advantages and disadvantages were discussed to provide a perspective for future studies.

## AFP immunoassay sensors

### *Sandwich-type immunosensors*

The sandwich-type immunoassays are based on the incorporation of capture antibody (Ab1) and detection antibody (Ab2), immobilized on the well and detection

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tag, respectively, to arrange a sandwich-type complex with a targeted biomarker.[22] Because of the coupling match antibody system, sandwich-type immunosensors show some crucial benefits including simple automation, time-effective detection, and high sensitivity and specify.[24] In addition, due to the low electrochemical activity of some biomarkers like AFP, there is a need to apply other nanomaterials to achieve high conductivity. In order to improve sensitivity, Yuan et al proposed the $Fe_3O_4$@Au-based pseudo-homogeneous immunosensor for AFP detection. Both magnetic nanoparticles and gold nanoparticles which acted as carriers, show a critical role for signal amplification due to their excellent surface area and high biocompatibility. The antibodies were immobilized on the $Fe_3O_4$@Au NPs and horseradish peroxidase with Au NPs as the capture tag and signal-amplifying label, respectively. After the formation of the immunocomplex, decomposition of the $H_2O_2$ (via horseradish peroxidase catalyze effect) causes the current substantial for AFP detection. The amperometric response demonstrates the dynamic range from 20 to 100 ng/mL with an LOD of the 0.64 ng/mL.[24] In another AFP electrochemical immunoassay study, Zhang et al modified the glassy carbon electrode (GCE) via polyaniline nanofiber which demonstrates high sensitivity to Cd(II) and Pb(II) ions, as a sandwich-type electrogenerated chemiluminescence sensor for simultaneous detection of some cancer biomarkers like AFP. In this study, metal-organic framework (MOF) of the ions were prepared in combination with 2-aminoterphthalic acid and utilized as electrochemical labels for secondary anti-AFP antibody. This platform demonstrates a linear response in a range of 0.3 to $3 \times 10^3$ pg/mL with the LOD of 0.1 pg/mL.[25]

### *Polymer-based immunosensors*

Conducting polymeric-based nanoparticles are of great significance for the electrochemical detection of analytes due to their high electrochemical activity and biocompatibility.[26] Polypyrrole (PPy) is one of these polymeric NPs which is well-known for its rapid electron transferring, excellent surface-to-volume ratio and good stability. Nevertheless, the most challenging issue for use of PPy is its individual dispersion.[27] Xu et al developed an electrochemical immunosensor based on the cooperation of PPys with γ-polyglutamic acid (PGA) and heparin (Hep) on the surface of the GCE for AFP detection. The use of PGA is related to its high biocompatibility and Hep is utilized as an anticoagulant agent for detection in whole blood medium. As illustrated in Figure 1, by immobilizing anti-AFP on the surface of PPys-PGA-Hep NPs, the anti-biofouling electrochemical biosensor was designed and the detection mechanism was related to redox activity of $[Fe(CN)_6]^{3-/4-}$ during the protein layer formation on the surface of GCE. Finally, the biosensor was applied for determination of AFP in whole blood samples. Whole blood is a complex matrix which influence the detection approach, but it is valuable; because serum samples waste lots of time for preparation and centrifugation steps.[13,16,28] The developed biosensor detects AFP in the dynamic range of 0.1-100 ng/mL with an limit of detection (LOD) of 0.099 ng/mL.[29]

Recently, hyperbranched polymers have been introduced as an effective material for biosensor modifications due to their numerous active terminal functional groups which are disposed to further functionalization.[30] For example, poly-glycerol with a hyperbranched globular form is used for the fabrication of a novel biosensor reported by Ma et al. The advantages of the hyperbranched poly-glycol (HPG) are related to their excellent antifouling activity. The antifouling activity is the key property of electrochemical biosensors not only due to the prevention of false signals and reducing background interference but also for possessing the ability of target binding ability.[31] In order to increase the conductivity of the HPG, Ma et al. functionalized poly(3,4-ethylenedioxythiophene) (PEDOT) with excellent transparency with HPG and

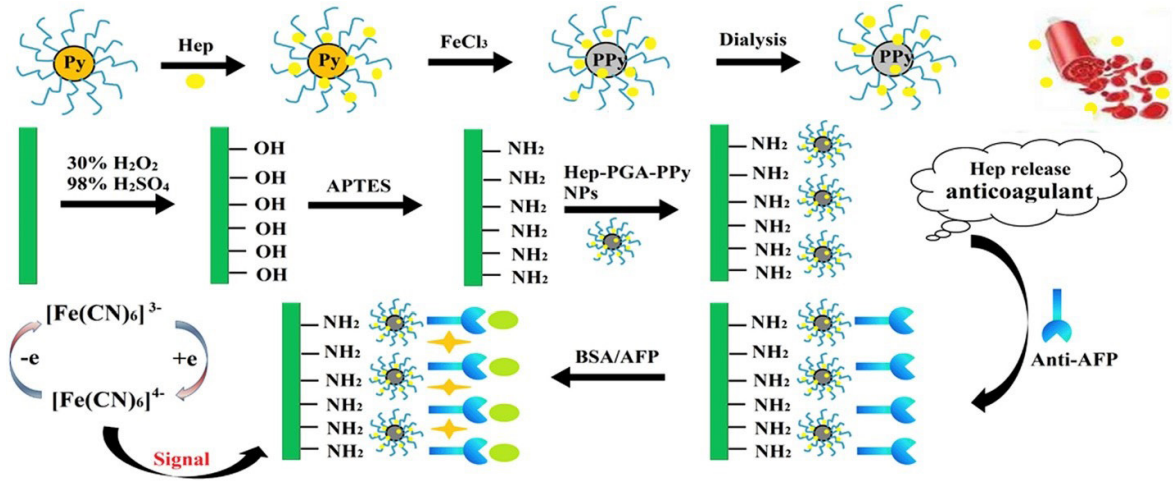


**Figure 1.** The schematic illustration of the fabrication of the Hep-PGA-PPy NPs based immunosensor for detection of AFP reported by Xu et al.[29] (License Number: 5436760749340)

conjugated to the AFP antibody on the surface of the GCE. The mechanism of action of the fabricated immunosensor is illustrated in Figure 2. The linear relationship was observed in the range of 0.10 pg/mL to 1.0 ng/mL.[32]

Some synthetic or natural polymers called dendrimers have been considered significantly due to their branched structure with several functional groups which made an appropriate platform for binding to biological targets. Chikhaliwala et al utilized hyperbranched amino functionalized (PAMAM) dendrimers to introduce a novel immunosensor for AFP determination. In fact, the magnetic nanoparticles ($Fe_3O_4$ NPs) were modified by PAMAM dendrimers which not only made high surface area, but also PAMAM dendrimers act as a stabilizer that prevents the aggregation of $Fe_3O_4$ NPs. Then NP-conjugated antibodies and redox dye (Prussian blue) were decorated on the surface of GCE. The antibody-antigen complex formation decreases the current during the dual differential pulse voltammetry (DPV) and detects the AFP in the linear range from 0.02 to 10 ng/mL with LOD of 50 pg/mL. Also, this method was applied for human serum sample analyses.[33] In another study, Niu et al reported the hyperbranched polyester NPs with nitrite groups (HBPE-$NO_2$) as a label-free immunosensor for AFP determination. Owning to the 3D architecture and some properties like the high density of end-groups, the cavity within molecules and the nanosized effect, hyperbranched polymers have been considered for sensing platforms. The linear dynamic range of the developed sensor was reported as 0.1-120 ng/mL with an LOD of 0.055 ng/mL.[34]

In other study, Liu et al fabricated an AFP immunosensor consist of Ag and Cu nanoparticles on polydopamine (PDA) functionalized cellulose nanofibrils (CNFs) through an amperometric method. CNFs/PDA cooperation was used as a substrate for Cu-Ag nanoparticles deposition by reducing agent. The AFP was quantified by $H_2O_2$ reduction in linear range of 0.01-100 ng/mL with LOD of 4.27 pg/mL.[35]

### *AFP aptasensors*

Owing to some advantages like intrinsic selectivity, good stability and reversible denaturation, aptamers have been provided a feasible alternative in developing various types of rapid sensing platforms in recent years.[36] A novel aptasensor for AFP detection was proposed by Cui et al which consisted of a long-chained AFP aptamer as recognition units and short-chained zwitterionic peptides immobilized on the surface of the Au electrode (Figure 3). It is noteworthy that zwitterionic peptides have the ability to coordinate an antifouling layer which leads to inhibiting of nonspecific adsorptions. Besides, using of peptides which are inherently natural biological structures possess the biocompatibility of the biosensor. In the present AFP sensor, AFP-aptamer complexation causes conformational changes and increases the charge-transfer resistance which subsequently leads to a decrease in the DPV signal. The detection process was confirmed in a linear range from 10.0 fg/mL to 100.0 pg/mL with an LOD of 3.1 fg/mL.[37]

Furthermore, Yang et al designed an AFP electrochemical aptasensor based on graphene oxide (GO) which is covalently bonded to the $NH_2$-functionalized AFP-specific aptamer through carboxylic groups. As an advantage of the use of GO, it has a large surface area with several COOH groups leading to effective binding with $NH_2$-functionalized aptamer. Also, it can prevent aggregation which made the analysis more sensitive. It is noteworthy to mention that this method can be more cost-

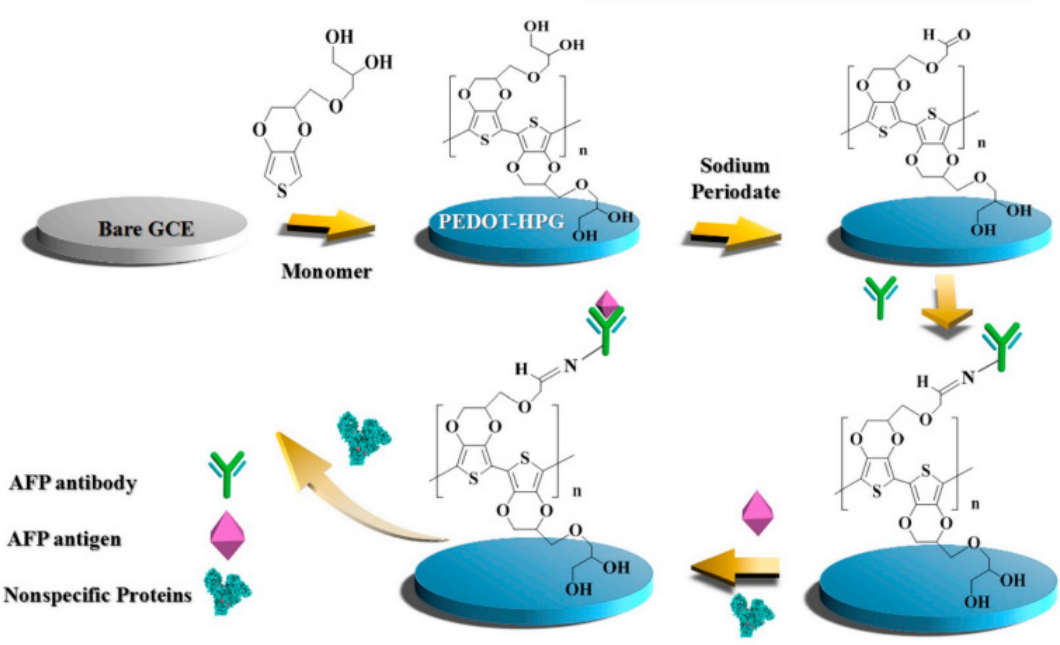


**Figure 2.** The schematic illustration of the process of the HPG-PEDOT based AFP biosensor reported by Ma et al[32] (License Number: 5436770295525)

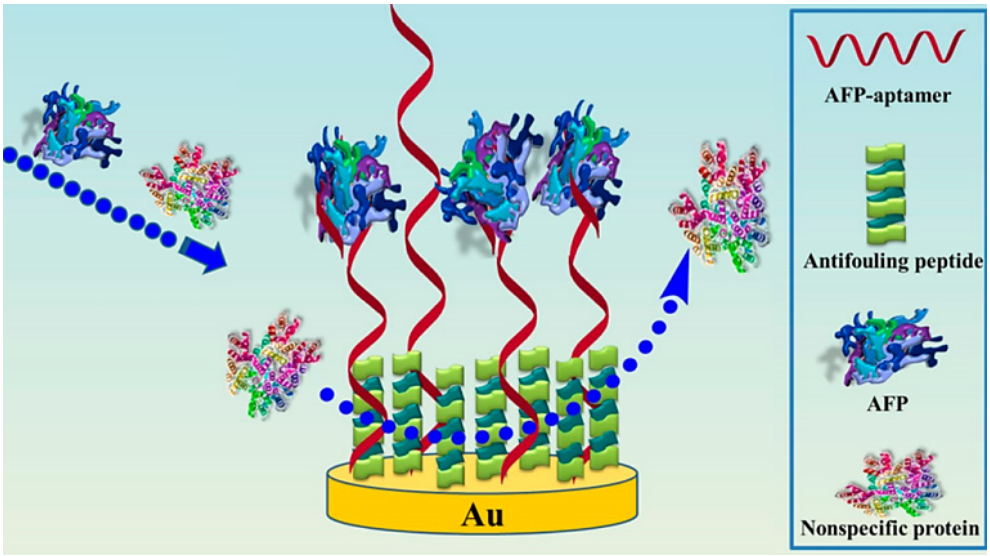


**Figure 3.** The schematic illustration of the AFP aptasensor reported by Cui et al[37]

effective in comparison to other methods like sandwich-type immunosensors which have several antibody labeling steps. The obtained results demonstrated that the cyclic voltammetry (CV) signals changed linearly in the AFP concentration from 0.01-100 ng/mL and the LOD was 3 pg/mL.[38] In another study, Upan et al utilized carboxylated-GO for modification of the platinum NPs (PtNPs/GO-COOH) to fabricate the aptasensor for electrochemical detection of AFP. The PtNPs/GO-COOH was screen-printed on the surface of the graphene-carbon paste electrode and employs as the immobilization element. PtNPs enhance the electrical conductivity and increase the current response of hydroquinone that was used as a redox probe. Also, the AFP aptamer, as the specific bio-recognition unit, was decorated on the modified electrode. The aptamer-AFP interaction decreases the hydroquinone current signal and provides the linear relationship in the concentration range of 3.0–30 ng/mL AFP with the LOD of 1.22 ng.mL$^{-1}$.[39]

### *MIP-based AFP sensors*

The combination of MIP platforms with electrochemical sensors is a great deal in recent analytical sensing studies to provide artificial receptors via a polymer network around a template structure which is simple, cost-effective, and specific through the fitting for its printed molecules.[40] It is worth mentioning that MIP-based sensors are not commonly applied for small molecules because of the complex structure of biomacromolecules. In this way, Shen et al developed an electrochemical MIP-based sensor by direct surface imprinting method on the GCE for AFP detection. This process started via modification of the GCE through layer-by-layer coating of chitosan and glutaraldehyde, and then was completed by the polymerization of acrylamide to arrange a narrow polymer film on the electrode surface. Here chitosan is acted as an electron transfer enhancer and glutaraldehyde has a role in the grafting of AFP for the polymerization step (Figure 4). The obtained results demonstrated the linear relationship of the current peak and AFP concentration in 0.8 ng/mL to 10 μg/mL and the LOD was calculated 0.096 ng/mL.[40]

In another study, Ouyang et al developed a MIP-coated printed carbon electrode that incorporated with biofuel cells using 4-aminophenylboronic acid/CNT/bilirubin oxidase nanocomposite (APBA/CNT/BOD). While the MIP part acts as the recognition unit, the APBA/CNT/BOD was used as the signal probe and biocatalyst part. The developed sensor acts through the reduction of the oxygen in air-saturated phosphate-buffered saline after the AFP binding signal immerses the electrode in APBA/CNT/BOD solution. This part constructed the biocathode and bioanode was organized via thionine/graphene/glucose dehydrogenase carbon electrode which worked over the glucose oxidation. Using the CV analysis, the AFP was determined in the dynamic range of 1 ng/mL to 1 mg/mL and the LOD was 1 ng/mL.[41]

## Conclusion

All of these technologies provide a beneficial detection platform that is fast and cost-effective in comparison to other analytical methods for on-site applications. Some of these methods with analytical data are classified in Table 1. Individually, immunosensors based on the interaction of the antibody and antigen (AFP) are discussed in this review in two main groups of sandwich-type and polymer-based AFP immunosensors. Both of them are specific with high accuracy platforms. They need several time-consuming antibody labeling steps, especially sandwich-type biosensor (based on the incorporation of capture antibody (Ab1) and detection antibody (Ab2), immobilized on the well and detection tag, respectively, to arrange a sandwich type complex with targeted biomarker). Aptasensors consist on the

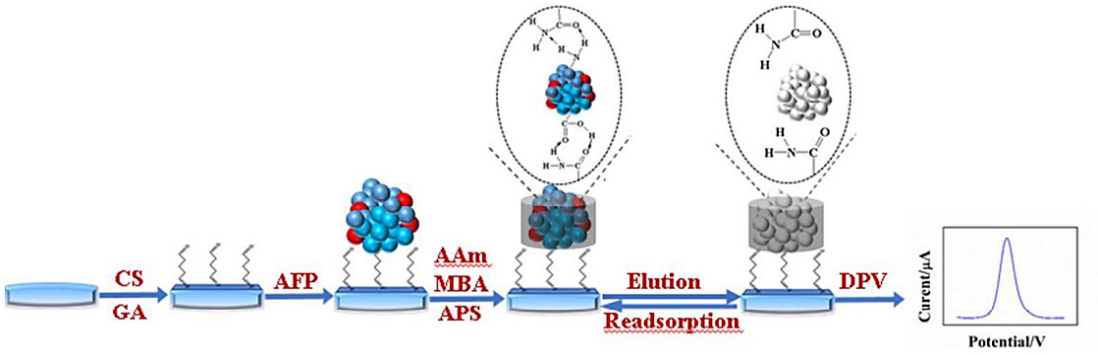


**Figure 4.** The schematic illustration of the MIP-based sensor for AFP detection reported by Shen et al[40]

**Table 1.** The analytical properties of some reports for determination of AFP

| Classification | Materials | LOD | Linear range | Ref. |
|---|---|---|---|---|
| Immunosensor | Polydopamine (PDA) functionalized cellulose nanofibrils- Ag-Cu nanoparticles | 4.27 pg/mL | 0.01-100 ng/mL | [35] |
| MIP-based sensors | GCE/CS/ glutaraldehyde (GA)/MIP | $9.6\times10^{-5}$ μg/ mL | $8.0\times10^{-4}$ -10 μg/ mL | [40] |
| Immunosensor | Ab/CuFC-C/GCE | $40\times10^{-8}$ ng/mL | - | [42] |
| Immunosensor | AB/Ni-Co MOF nanosheets/ polyethylene terephthalate (GNP) electrode | 0.3 ng/mL | 1–200 ng/mL | [43] |
| Immunosensor | Ab-Au nanoparticles- $Cu_2O$@$MoS_2$) nanohybrid- quartz crystal microbalance electrode | 35 pg/mL | - | [44] |
| Immunosensor | Ab- polyethyleneimine -$Cu_2S$@$Co_3S_4$ nanosheets | 0.6 pg/mL | 1 pg/mL - 100 ng/mL | [45] |
| Aptasensor | Au/ hairpin-structured DNA-MB | 8.76 pg/mL | 50 pg/mL - 10 ng/mL | [46] |
| Aptasensor | Phenylboronic acid- Aptamer | 0.037 ng/mL | 0.1 to 100 ng/mL | [47] |
| Aptasensor | Au electrode/thiolated aptamer/ MB- oligo-DNA-tagged anti-AFP | 1 pg/mL | 2 pg/mL - 20.0 ng/mL | [48] |

interaction between aptamer and AFP have advantages through intrinsic selectivity, good stability, and reversible denaturation which are known as feasible alternatives in developing various types of rapid sensing platforms in recent years. At last, MIP-based platforms which are constructed by the artificial receptors via a polymer network around a template structure are simple, cost-effective, and specific through the fitting for its printed molecules. Developing a new electrochemical platform for AFP detection is a great deal in recent years due to the importance of the AFP in the early-stage diagnosis of HCC. By the way, more research is needed to achieve a sophisticated approach that is more sensitive and selective and can be applied as the point-of-care continuous monitoring device for on-site monitoring applications.

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## Author Contributions
ZG: Writing original draft; JS: Reviewing; Conceptualization and approval of the manuscript. All authors read and approved the manuscript.

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## Ethics Issues
Not applicable.

## Conflict of Interests
There is no conflict of interests.

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