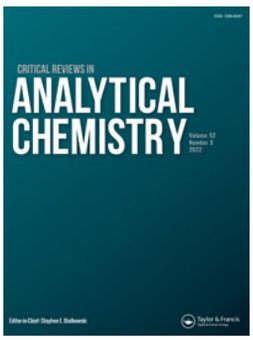

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Optical and Electrochemical Aptasensors Developed for the Detection of Alpha-Fetoprotein

Dinh Tran Ngoc Huy[a,b], A. Heri Iswanto[c], Maria Jade Catalan Opulencia[d], Fahad Al-Saikhan[e], Anton Timoshin[f], Azher M. Abed[g], Irfan Ahmad[h], Sofiya A. Blinova[i], Ali Thaeer Hammid[j], Yasser Fakri Mustafa[k], and Pham Van Tuan[l]

[a]MBA, Banking University HCMC, Ho Chi Minh City, Vietnam; [b]International University of Japan, Niigata, Japan; [c]Public Health Department, Faculty of Health Science, University of Pembangunan Nasional Veteran Jakarta, Jakarta, Indonesia; [d]College of Business Administration, Ajman University, Ajman, United Arab Emirates; [e]Department of Clinical Pharmacy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Alkharj, Kingdom of Saudi Arabia; [f]Department of Propaedeutics of Dental Diseases, I.M. Sechenov First Moscow State Medical University (Sechenov University), Moscow, Russia; [g]Department of Air Conditioning and Refrigeration, Al-Mustaqbal University College, Babylon, Iraq; [h]Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid University, Abha, Saudi Arabia; [i]Department of Histology, Embryology, and Cytology, Samarkand State Medical Institute, Samarkand, Uzbekistan; [j]Computer Engineering Department, Imam Ja'afar Al-Sadiq University, Baghdad, Iraq; [k]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq; [l]National Economics University (NEU), Hanoi, Vietnam

ABSTRACT

Early diagnosis of hepatocellular carcinoma (HCC), a leading cause of cancer mortality, is decisive for successful treatment of this type of cancer and increasing the patients' survival rate. Alpha-fetoprotein (AFP) is a glycoprotein that has been currently employed as a potential serological biomarker for determination of HCC and several other cancers. Achieving highly sensitive and specific detection of this biomarker is an effective strategy to inhibit developing issues caused by the cancer. Though, traditional procedures cannot meet the requirements due to the technical drawbacks. Recently, growing number of aptamer-based biosensors (aptasensors) attracted important attention as superior diagnostic tools because of their unique properties such as high stability, target versatility and remarkable affinity and selectivity. Nanomaterials, which broadly employed in the structure of these aptasensors, can considerably enhance the detection limit and sensitivity of analytes determination. Therefore, this review selectively investigated the recent progresses in several different optical and electrochemical aptasensors and nano-aptasensors designed for AFP assay.

KEYWORDS

α-fetoprotein; aptamer; aptasensing; biosensor; cancer biomarker

1. Introduction

According to World Health Organization (WHO), approximately 10 million deaths occurred in 2020 from different types of cancers.[1] Minimizing delays to treatment of cancer, enhance the chance of effective therapy and quality of life in these population.[2,3] The quantification of blood-based tumor markers has appeared as a potential medical tool for rapid diagnosis, condition monitoring and therapeutic assessment of cancer in these patients.[4]

Alpha-fetoprotein (AFP) has been postulated as an useful tumor marker for diagnosis of hepatocellular carcinoma (HCC), one of the leading causes of cancer deaths.[5] Furthermore, it has currently been utilized in clinic as a specific biomarker for detection of other various types of human cancers such as gastric, ovarian, breast, testicular and pancreatic tumors as well as pulmonary and Barrett's adenocarcinoma.[6] AFP is an oncofetal glycoprotein of 70 kDa that is mainly produced by the yolk sac and fetal liver and decreases rapidly to normal values by the second year of life.[7] The concentration of AFP in serum of healthy people is considered to be lower than 5–8 ng/mL, while the higher serum levels of AFP above the normal values can be a sign of cancer.[6,8]

Currently, a series of traditional techniques have been established for AFP detection including enzyme-linked immunosorbent assay (ELISA),[9] electrochemical immunoassay,[10] radioimmunoassay (RIA) and immunoradiometric assay (IRMA),[11] as well as fluorescence,[12] colorimetric, chemiluminescence and electrochemiluminescence immunoassays.[13] Nevertheless, these methods are time-consuming, inconvenient and usually need complicated instruments, costly reagents and professional operating.[14–16] Additionally, variation of antibody affinity and not sufficient sensitivity have made these approaches unreliable to precise detection in clinic.[17] Hence, demands grow progressively for early, cost-effective, highly sensitive and specific detection of AFP in the serum of different human cancers. Implementation of biosensor-based methods is probably one of the fastest growing areas that solve difficulties regarding efficient, sensitive, rapid, real-time and cheap detection.[18]

CONTACT Pham Van Tuan Phamvantuan@neu.edu.vn National Economics University (NEU), Hanoi, Vietnam.

Table 1. Comparing the benefits of using aptasensors over immunosensors.

Advantages of aptasensors	Disadvantages of immunosensors
• Superior analytical performance • Enhanced stability • Versatile design • Ability to be regenerated for reusable biosensors • Significantly longer shelf life • Can be stored for several weeks without sensitivity loss • Regeneration can be easily achieved without hampering their activity • Aptamer's small size allows their immobilization in a dense receptor layer on the biosensor surface • Better stability, simplicity and cost effectiveness.	• Storage is usually limited to several days • Regeneration is more challenging • Antibodies are less stable in adverse environmental conditions, such as pH, ionic strength, and temperature

Biosensors are integrated receptor-transducer devices, which are able to convert biological responses into electrical signals and provide selective quantitative or semi-quantitative analytical data.[19] Based on their transducers, biosensors have been grouped in two main classes including optical and electrochemical.[20] Optical biosensors pose superior benefits including high specificity, sensitivity and low expenses as well as their capability in real-time monitoring and label-free determination.[21] The electrochemical aptasensors are also great appropriate tools for rapid detection of biomarkers because of their simplicity, high sensitivity and selectivity as well as cost-effectivity, and portability.[22,23] Application of nanomaterials (NMs) in the structure of biosensors can considerably improve their sensitivity and biomarker detection ability.[24] Combination of NMs and various bio-recognition elements, especially aptamers, antibodies, DNA and enzymes can also noticeably enhance the performance of biosensor system for better quantification of cancer-specific markers.[25,26]

As indicated in Table 1, aptamers exhibited great features in comparison with antibodies including great sensitivity, stability, lower costs, and easy production which have made them appropriate choices for construction of AFP aptasensors. Aptamers are single-stranded 15–40 bases long nucleic acids generated by a combinatorial strategy called Systematic Evolution of Ligands by Exponential enrichment (SELEX).[27] Aptamers pose unique features for bioanalytical analysis including ease of synthesis, target-induced conformational changes, low molecular weight, target versatility, remarkable affinity and selectivity, resistance to degradation and denaturation, and low production costs.[2] Accordingly, these advantages have led to the development of various aptamer-based biosensors (aptasensors) for AFP detection.

To our best knowledge, the current study is the first review article presents an overview of several optical and electrochemical aptasensors established for AFP assay. We further highlighted the significant role of nanomaterial-based aptasensors (nano-aptasensors) in the higher sensitive and specific detection of AFP. Additionally, the developed aptasensors were critically compared with the commercially available kits applied for AFP detection.

2. Aptamers selected against AFP

For development of extremely sensitive and specific aptamer-based biosensors for AFP assay, selection of AFP-targeting aptamers has a vital role. Accordingly, a number of articles have been studied about the characterization and selection of AFP aptamers (Table 2). For example, Lili Dong et al. used capillary electrophoresis (CE)-SELEX technique for efficient screening of a ssDNA aptamer (AP273) which demonstrated superior specificity and high affinity ($K_d = 0.5\ \mu M$) toward AFP biomarker.[28] Besides, in another study by Chao-Jyun Huang and coworkers, an integrated microfluidic system was employed for rapid selection of ssDNA aptamers against AFP. One of these aptamers that had the highest affinity ($K_d = 2.3$ nM), applied for AFP detection and indicated a linear detection range from 12.5 to 800 ng/mL.[29] Moreover, an AFP-specific RNA aptamer was screened using SELEX method with a great affinity of 33 nM. This aptamer efficiently suppressed HCC proliferation and the oncogene expression.[30]

3. Optical AFP aptasensors

Recently, optical biosensing devices have shown great promise to be used as medical diagnostic tools for detection of biomarkers.[31] These biosensors offer great advantages due to their ability in real-time and label-free quantification, as well as high specificity, sensitivity and low costs.[26]

Growing evidence have established that application of NMs as signal amplifiers is necessary for construction of ultrasensitive biosensors and enhancing the limit of detection (LOD) of target.[32,33] As demonstrated in Table 3, increasing studies have recently fabricated effective optical aptasensing platforms including luminescence, fluorescence and liquid crystals as well as surface-enhanced Raman spectroscopy, light scattering and some other means for AFP quantification which are briefly discussed in the following section.

3.1. Luminescent AFP aptasensors

Luminescence is spontaneous light emission by certain materials without heat generation.[49] There are different

Table 2. Available aptamers specifically for AFP assay.

Aptamer type	Sequences	Affinity (K_d)	References
ssDNA	TCAGGTGC. AGTTCTCGACTCGGTCTTGATGTGGGT	0.5 μM	[28]
ssDNA	GGCAGGAAGACAAACAAGCTTGGCGGCGGGAAGG TGTTTAAATTCCCGGGTCTGCGTGGTCTGTGGTGCTGT	2.37 nM	[29]
RNA	GGGGGGATCCATCGACCTCTGGGTTATG	33 nM	[30]

kinds of luminescence that can be classified by the energy source such as chemiluminescence (CL) in which light is produced by chemical reactions. Nonetheless, light is emitted via electrochemical reactions in electrochemiluminescence (ECL).[50] This technique is especially emerging because of its capability in high sensitive and selective detection of analytes.[51]

In a recent study conducted by Rui Han et al., a simple CL aptasensing platform was fabricated for detection of trace levels of AFP by employing an iron-based metal organic framework (Fe-MOF) as signal amplifier. The addition of AFP and subsequent aptamer conformational alternation resulted in production of CL signal proportional to the analytes concentration. This aptasensing platform provided a LOD of 7.7×10^{-11} g/mL with a wide linear range of 10^{-10} to 3×10^{-5} g/L.[34]

Moreover, CL technique was used for detection of AFP in a biosening platform using aptamer-coated magnetic beads (MBs). This aptasensor demonstrated a LOD of 12.5 ng/mL with a wide linear range of 12.5 to 800 ng/mL.[29]

Mei-Sheng Wu et al. also constructed a novel ECL biosensor for simultaneous AFP assay based on closed bipolar electrode (BPE) array. They used aptamer or antibody as biorecognation elements and thionine silica nanoparticles (Th@SiO2 NPs) as electrochemical tags. According to the results, this platform could detect AFP in a linear range of 0.07 to 14 ng/mL.[35]

3.2. Fluorescent AFP aptasensors

Fluorescence, a particular type of luminescence, has also gained great attention for biomarker quantification because it exhibits high sensitivity, signal production, reproducibility and simplicity and nondestructive outcomes.[14,52] In general, there are two approaches for construction of fluorescence aptasensors. In 'signaling aptamers', the aptamers are directly labeled with fluorophores, however, the aptamers are linked to fluorescence quenching molecules in fluorescence resonance energy transfer (FRET) technique.[27]

A FRET aptasensor for highly sensitive and selective detection of AFP was introduced by Guiyin Li et al. using palladium nanoparticles (PdNPs) as quencher and AFP aptamer labeled with 5-carboxyfluorescein (FAM). The LOD of this method was reported to be 1.4 ng/mL.[39]

In recent decades, gold nanoclusters (AuNCs) have gained researcher attention as donors in FRET biosensing because of their beneficial properties such as good biocompatibility and photo-stability.[53,54] Shenghao Xu et al. reported a 'switch on' FRET-based aptasensor for dual color simultaneous detection of AFP and carcinoembryonic antigen (CEA) with a single wavelength excitation. To fabricate this aptasensor, molybdenum disulfide (MoS2) nanosheets, as promising quenchers, were combined with green colored AFP aptamer-$AuNCs_{510\,nm}$ and red colored CEA aptamer-$AuNCs_{650\,nm}$. This aptasensor demonstrated a LOD of 0.16 ng/mL for AFP and 0.21 ng/mL for CEA.[43]

Ya Zhang et al. also successfully designed a simple and cost-effective fluorescent aptasensor for ultrasensitive AFP assay employing FAM-aptamer/GO. Briefly, by addition of AFP, interaction between the FAM-aptamer and AFP resulted in the conformational change of FAM-aptamer, release from the GO surface and fluorescence restoration. The produced aptasensor showed an extraordinary LOD of 43 fM in the detection range of 0 to 10 pM.[42]

Lu Zhou and coworkers fabricated another simple and sensitive aptasensor that could detect AFP using FRET system (Figure 1). The aptamers were immobilized covalently on luminescent CdTe quantum dots (QDs) and employed as donors. AuNPs were also functionalized with anti-AFP antibodies and used as acceptors. When the target was added to this system, the specific binding of aptamer, AFP, and antibody made the QDs and AuNPs close enough to initiate FRET and suppressed fluorescence intensity based on the AFP concentration. The LOD and linear range of the aptasensor was determined to be 400 pg/mL and 0.5–45 ng/mL, respectively.[40]

In another recent work presented by Shengqiang Li and coworkers, an enzyme-free platform designed for highly sensitive fluorescence AFP assay based on aptamer-mismatched catalytic hairpin assembly (MCHA) system which provided an efficient low-background cascade signal amplification. As shown in Figure 2, upon target AFP introduction, the triggers released and initiated to form H1 and H2 double chain compound. Afterwards, this complex reaction with F@Q resulted in the separation of fluorescence quenched Q chain. In this assay, the linear range and LOD were 0.1 ng/mL to 10 µg/mL and 0.033 ng/mL, respectively.[55]

Graphene oxide (GO) is a highly studied nanomaterial which has extraordinary potential in quenching fluorescence of many fluorophores.[56] In this regard, Jiayao Xu et al. fabricated an effective and highly specific four-color fluorescent aptasensor for simultaneous determination and imaging of multiple tumor-associated biomarkers including AFP, vascular endothelial growth factor-165 (VEGF165), CEA and human epidermal growth factor receptor 2 (HER2) in living cells. Based on the achieved data, the developed aptasensor had LOD as low as 0.45, 0.30, 0.62 and 0.96 nM for AFP, VEGF165, CEA and HER2, respectively.[38]

Similarly, Linshun Xie et al. have simultaneously detected several tumor biomarkers including AFP, CEA and carbohydrate antigen 125 (CA125) using an fluorometric microfluidic chip electrophoresis (MCE) aptasensing platform. Catalyzed hairpin assembly (CHA) used as amplification

Table 3. Optical aptasensors for AFP detection.

Detection methods	Strategy/nanomaterial	Aptamer type	Aptamer sequence	Limit of detection (LOD) (ng/ml)	Linear range (LR)/ (ng/ml)	Recovery rate in human serum samples (%)	References
Chemiluminescence (CL)	Fe-MOFs	–	–	0.077	0.1–30,000	98.9–104.1	[34]
CL	Apt-coated magnetic beads	DNA	GGCAGGAAGACAAA CAAGCTTGGCGGCGGGAAGGTGTT TAAATTCCC GGGTCTGCGTGGTCTGTGGTGCTGT	12.5	12.5–800	–	[29]
Electrochemiluminescence (ECL)	Th@SiO2 NPs	DNA	–	–	0.07–14	–	[35]
Fluorescent	Fe3O4@AuNPs	–	–	0.0001	–	88–104	[36]
Fluorescent	PDAN@AgNCs	DNA	TCAGGTGCAGTTCTCGACTCGGTCTTGATG TGGGTAAAAACCCCCTAATTCCCCC	165	686.8–6868	–	[37]
Fluorescent	GO	DNA	–	30	55–10,988	–	[38]
Fluorescent	FAM-AFP apt-PdNPs FRET	DNA	GTGACGCTCCTAACGCTGA CT CAGGTGCAGTTCTCG ACTCGGTCTTGATGTGGGT CCTGTCCGTCCGAACCAATC	1.4	5–150	98.3–112.9	[39]
Fluorescent	CdTe QD/ AuNPs	–	GGCAGGAAGACAAACAAGCTTG GCGGCGGGAAGGT GTTTAAATTCCCGGGTCTGCGTGGTCTGTGGTGCTGT	0.4	0.5–45	98.9–104.1	[40]
Fluorescent	MCHA/ H1@H2/ F@Q	DNA	GTGACGCTCCTAACGCTGACT CAGGTGCAGTTCTC GACTCGGTCTTGATGTGGGTCCTGTCCGTCCGAA CCAATC	0.033	0.1–10,000	–	[22]
Fluorescent	polyfluorene-based CPEs	DNA	GGCAGGAAGACAAACAAGC TTGGCGGCGGGAAGGTGTT TAAATTCCCGGGTCTGCGTG GTCTGTGGTGCTGT	1.76	10–800	–	[41]
Fluorescent	FAM-apt/GO	DNA	GTGACGCTCCTAACGCTGA CTCAGGTGCAGTTCTCGAC TCGGTCTTGATGTGGGTCC TGTCCGTCCGAACCAATC	0.003	0–0.686	95–102	[42]
Fluorescent	AuNCs-MoS2	DNA	GGCAGGAAGACAAACAAGC TTGGCGGCGGGAAGGTGTTT AAATTCCCGGGTCTGCGTGG TCTGTGGTGCTGT	0.16	0.5–60	98.17–102.14	[43]
Fluorescent	FAM-AFP apt-AuNCs FRET	DNA	GTGACGCTCCTAACGCTG ACTCAGGTGCAGTTCTCGA CTCGGTCTTGATGTGGGTCC TGTCCGTCCGAACCAATC	6.631	10–100	–	
Liquid crystals (LCs)	–	–	–	0.01865	0.02–0.8	–	[44]
LCs	MBs/apt1-target-apt2	DNA	–	0.19	–	–	[45]
SERS	DNA functionalized hydrogels	DNA	TGACGCTCCTAACGCTGA CTCAGGTGCAGTTCTCGA CTCGGTCTTGATGTGGGT CCTGTCCGTCCGAACCAATC	0.05	0.05–500	84.5–104.7	[46]
Resonance light scattering (RLS)	cDNA1 hybridization with cDNA2/ methyl violet	DNA	TCAGGTGCAGTTCTCGAC TCGGTCTTGATGTGGGTTG GAGTCTGAGAAAGGAGTTTG	0.94	50–100	91–109	[47]
Light-addressable potentiometric sensor (LAPS)	AuNPs	DNA	TCAGGTGCAGTTCTCGACT CGGTCTTGATGTGGGTCCC CCC	92	100–100,000	95.92–125.54	[48]

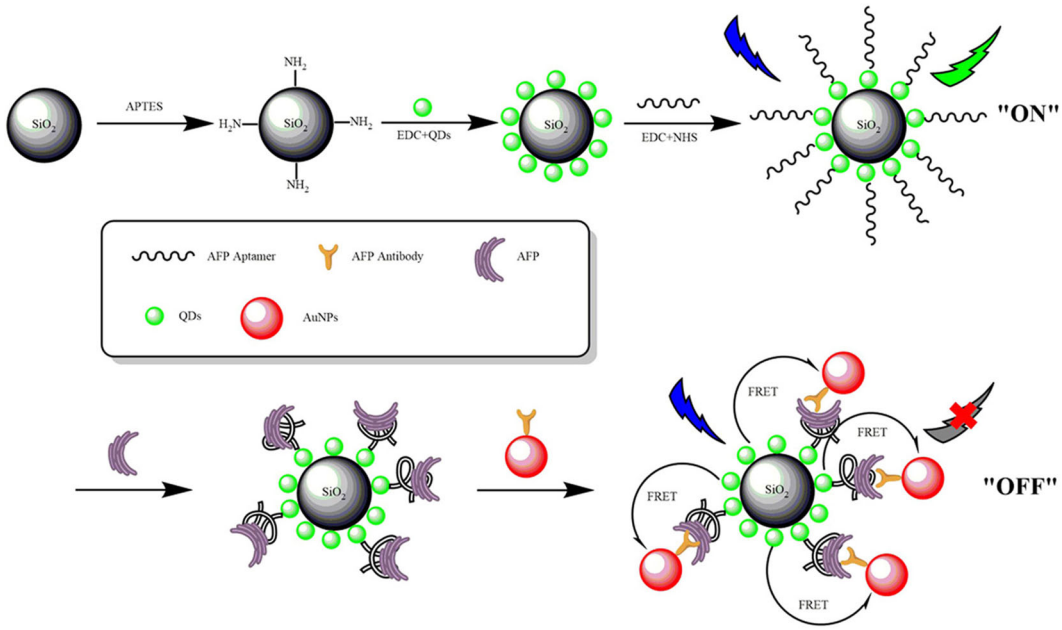

Figure 1. Schematic illustration of a FRET based sandwich structured QDs-AFP-AuNPs aptasensor for AFP detection. Reprinted with permission from Zhou et al.[40]

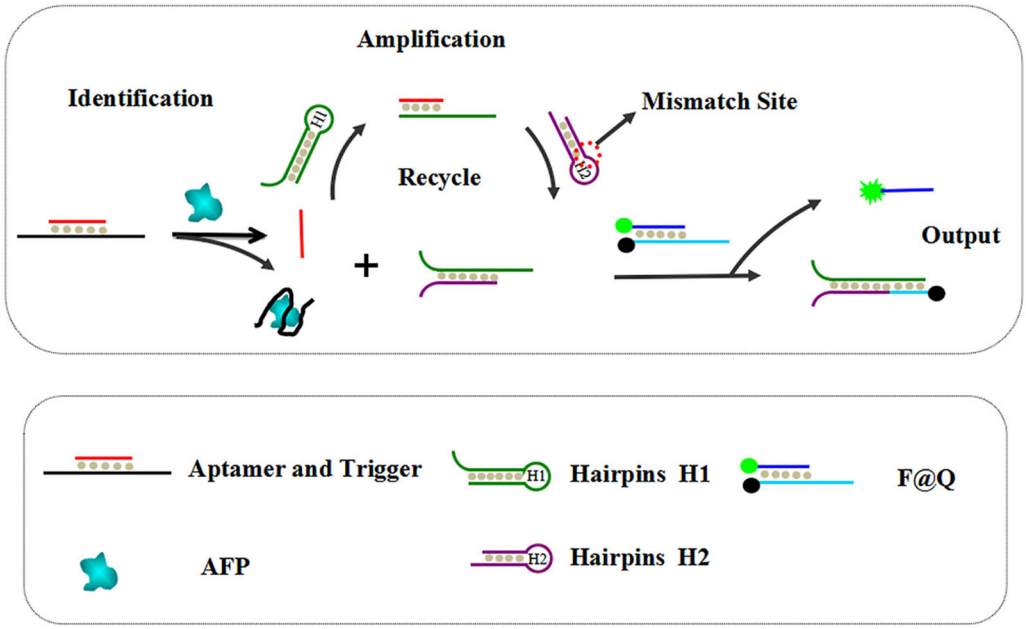

Figure 2. Schematic illustration of fluorescence sensing strategy based on aptamer recognition and mismatched catalytic hairpin assembly for highly sensitive detection of AFP. Reprinted with permission from Li et al.[55]

and the aptamers were covalently attached to Fe3O4@AuNPs MBs as capture probes. Afterwards, their antibodies-biomarkers labeled with DNA primers as signal tags and antibody-biomarker-aptamer complexes were formed. This rapid and cost-effective biosensor could simultaneously detect AFP, CEA and CA125 with LOD of 0.1, 0.2, 0.15 pg/mL, respectively.[36]

Silver (Ag) NCs are also ideal options for application in optical fluorescent biosensors because of their unique features including high quantum yield, ease of production, and tunable fluorescence emission.[57,58] Therefore, one group have designed a fluorescent aptasensor based on Polydopamine nanosphere (PDAN)@AgNCs for simultaneous determination of AFP and CEA with LOD of 2.4 nM and 5.6 nM, respectively. The AgNCs with different emissions were fabricated by two different aptamers and attached to the surface of PDAN, as quencher, by π-π stacking. Upon addition of the analytes, the formation of aptamer-analyte complexes resulted in the release of AgNCs from the surface of PDAN and recovery of fluorescence. The concentrations of the analytes were demonstrated based on the peak intensities.[37]

In addition, fluorescent cationic conjugated polyelectrolytes (CPEs) have gained excessive attention due to their great signal amplification capabilities and potential uses in detecting biomarkers in disorders.[59,60] In a study, a simple

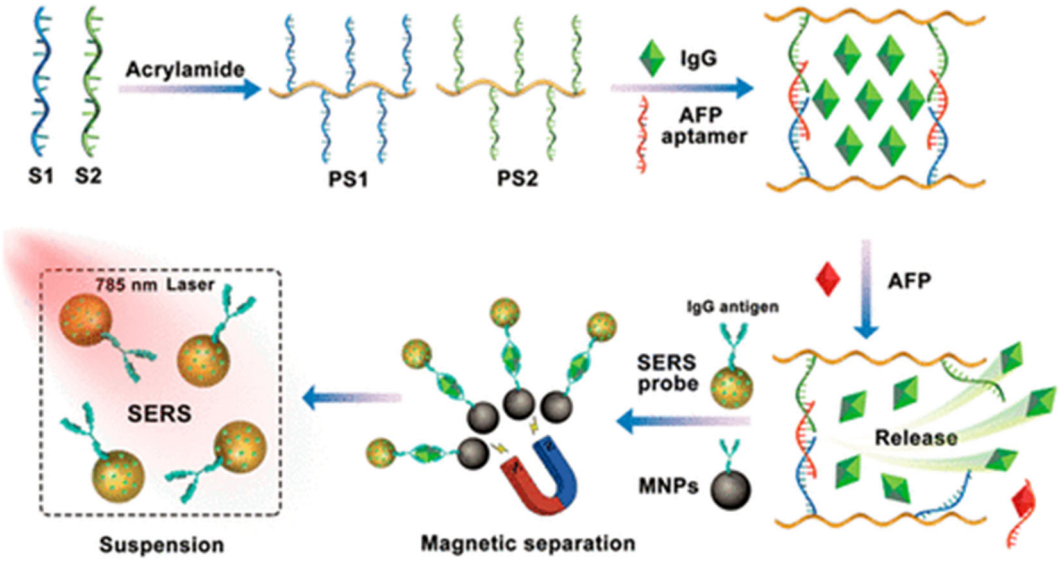

Figure 3. Schematic illustration of aptamer responsive DNA functionalized hydrogels for the sensitive detection of AFP using SERS method. Reprinted with permission from Wang et al.[46]

and label-free aptasensor employed polyfluorene-based CPEs as recognition probe for detecting AFP and CEA tumor markers. They suggested that this biosensor could offer a convenient and universal platform for the sensitive detection of analytes and a LOD of 0.316 and 0.176 ng/mL were achieved for CEA and AFP, respectively.[41]

3.3. Liquid crystals based optical AFP aptasensors

Liquid crystals (LCs) based biosensors have been emerged as excellent tools for detection of targeted analytes.[61] Taking advantages of LCs and aptasensors, some studies attempted to detect AFP.

For instance, a label-free LCs based assay targeting the detection of AFP was recently reported by Duong Song Thai Duong and Chang-Hyun Jang. The aptamer was immobilized on the surface of substrates and LC alignment was disrupted upon the addition of AFP. The proposed sensitive and specific aptasensor was capable of detecting the analyte with the LOD of 12.62 and 18.65 pg/mL in HEPES buffer and human serum, respectively and the concentration range was between 20 and 800 pg/mL.[44]

In another recent work, Lubin Qi and coworkers developed a highly sensitive LCs based aptasensor with target-induced dissociation of an aptamer. Aptamer1-coated MBs were employed for capturing tumor specific biomarkers including prostate specific antigen (PSA), CEA, and AFP in patients' serum and then a sandwich structure of aptamer1/protein/aptamer2 are formed on the MBs for high specific detection of targets which led to the release of signal DNA and induction of LCs sensors. The quantification limit of PSA, CEA and AFP was reached to 0.07, 0.31 and 0.19 ng/mL, respectively using this aptasensing platform.[45]

3.4. Other optical AFP aptasensors

Qi Wang and coworkers proposed an ultrahighly sensitive and cost-effective Surface Enhanced Raman Spectroscopy (SERS) based assay for AFP detection using target-responsive DNA hydrogel. According to Figure 3, the immunoglobulin G (IgG) embedded in hydrogel was released in the presence of AFP and integrated with SERS probes and led to the signal of Raman tags declining in the supernatant following magnetic separation. The wide linear range was from 50 pg/mL to 0.5 μg/mL and the LOD of AFP was down to 50 pg/mL.[46]

Feng Chen and coworkers prepared a simple and label-free resonance light scattering (RLS) based aptasensor for sensitive detection of AFP and microRNA-122, as two potential biomarkers in HCC diagnosis. The platform constructed by means of electronic interaction between methyl violet (MV) and dsDNA (cDNA1 hybridization with cDNA2). By adding the AFP or microRNA-122, the RLS signal altered proportionally with their concentration. The proposed aptasensing platform reached a LOD of 0.94 μg/L for AFP and 98 pM for microRNA-122 and linear range of the method was 5 to 100 μg/L and 200 pM to 10 nM for AFP and microRNA-122, respectively.[47]

Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has demonstrated superior potential in clinical use due to its several advantages including high throughput, rapid quantification and direct detection of analytes.[62] Therefore, a nanostructure ratiometric biosensor, based on MALDI-TOF MS was designed by Zidan Wang et al for detection of insulin and AFP. In this research, aptamer and molecularly imprinted polymers (MIPs), as two antibody substitutes, were attached to magnetic nanoparticles by Au-S bonds (Mag Au@SH-aptamer@MIP). This aptasensing platform was able to detect insulin in human serum dilution with a LOD of 0.5 ng/mL. Besides, AFP was sufficiently detectable from 20 to 1000 ng/mL in human saliva dilution.[63]

A label-free aptasensor was also fabricated using AFP aptamer immobilized magnetic NPs (Fe3O4@SiO2) by avidin-biotin interaction and high-performance liquid chromatography (HPLC). The LOD was 0.27 μg/mL with a linear range of 50 μg/mL.[64]

Guiyin Li et al., also fabricated a novel and label-free aptasensor combined with light-addressable potentiometric sensor (LAPS) for direct AFP quantification. The aptamer was integrated to the surface of LAPS functionalized with AuNPs and detected AFP in a wide linear range from 0.1 to 100 μg/mL. Besides, the sensor exhibited quantification limit of 92 ng/mL.[48]

3.5. Brief overview of optical AFP aptasensors

In general, optical assay techniques such as luminescence, fluorescence, LCs, SERS, RLS and LAPS have been used for fabricating AFP aptasensing platforms that have prominent advantages like feasibility of quantitative investigations without any need to developed devices. Fluorescence aptasensors are the most common optical techniques for AFP detection because of their superior features such as simplicity and high sensitivity.[65] Furthermore, according to Table 3, two fluorescence aptasensors presented the highest and lowest sensitivity for AFP assay with a LOD of 0.0001 and 165 ng/mL.

4. Electrochemical AFP aptasensors

Electrochemical aptasensing platforms are diagnostic tools constructed by attaching aptamers as the biorecognition elements into electrochemical transducer surface to generate assessable electrical signals.[23,66] The electrochemical aptasensors are very applicable tools for rapid quantification of targeted biomarkers due to their simplicity, high sensitivity and selectivity, cost-effectivity, and portability.[67,68] In the following parts, we summarized several developed electrochemical aptasensors based on electrical impedance spectroscopy, voltammetry, and photoelectrochemical methods for AFP determination (Table 4).

4.1. Electrical impedance spectroscopy based AFP aptasensors

Due to their ability in high sensitive, selective and label-free detection, electrical impedance spectroscopy (EIS) aptasensors have been employed for AFP assay. A label-free and ultrasensitive electrochemical aptamer-based biosensing platform was fabricated using nitrogen-doped nanoporous carbon NMs isolated from various plant biomass for early detection of AFP. This platform showed several advantages including cost-effectivity, environment friendly, and strong binding ability to aptamers and provided a superior LOD of 60.8 and 61.8 fg/mL by using EIS and differential pulse voltammetry (DPV) methods, respectively with a wide linear range of 0.1 pg/mL to 100 ng/mL. They also demonstrated that this aptasensor could be used as promising approach in the analysis of real serum samples of cancer patients.[78]

Supramolecular gels with self-healable abilities have gained growing research interest due to their high purity and well-defined molecular structures.[82] In this regard, an electrochemical aptasensor was prepared for AFP assay using –SH modified core–shell structured guanosine-borate (GB-SH) nanofiber hydrogel immobilized on an Au electrode. Afterwards, aptamer against AFP attached to this platform and employed for the target quantification by EIS technique which showed high sensitivity and LOD of 0.076 pg/mL for AFP assay. The linear range of the detection of this biosensor was also expanded using ferrocene modified guanosine-borate (GB-Fc) hydrogels (0.0005–100 ng/mL).[70]

In another similar research, multifunctional self-healing guanosine-pyridine-4-boronic acid (G-PyB/KCl) hydrogel was employed for development of an electrochemical aptasensor for the sensitive and accurate detection of AFP. This aptasensing platform displayed a low LOD of 0.51 pg/mL and wide linear range from 0.001 to 0.5 ng/mL using EIS method.[83]

4.2. Voltammetry AFP aptasensors

According to Table 4, voltammetry based aptasensors, including cyclic voltammetry (CV), DPV, square wave voltammetry (SWV), and alternative current voltammetry (ACV) have been extensively applied for AFP detection.

Due to their large specific surface area and functional groups, GO nanosheets obtained great attention as biomolecule immobilization platform.[84] For instance, Shaohong Yang et al. designed a label-free electrochemical aptasensor by means of GO. In this work, AFP aptamers were immobilized on GO sheet surface and CV method was employed for AFP determination. The sensing platform showed a low LOD of 3 pg/mL and the authors proposed that this simple and cost-effective sensing platform could be a promising strategy for AFP detection in clinical samples.[71]

Xingdong Yang and coworkers established a bi-directionally amplified ratiometric electrochemical aptasensor for highly sensitive determination of AFP using exonuclease-assisted target recycling and methylene blue (MB)-DNA-AuNP probe (Figure 4). The authors proposed that this platform is an emerging electrochemical system for point-of-care diagnosis that reached an ultralow LOD of 269.4 ag/mL by ACV method.[69]

In 2018, a label-free electrochemical aptasensor targeting AFP was effectively constructed using thionin/reduced GO/Au (TH/RGO/Au) NPs modified screen-printed electrodes (SPE) as sensing platform. Then the aptamer electrostatically adsorbed on the surface of SPE via TH amino groups. The aptasensing system exhibited high sensitivity and selectivity with a LOD of 0.05 µg/mL for AFP assay based on DPV technique.[75]

Wenzhan Li et al. introduced another label-free electrochemical aptasensor for AFP assay through DPV method (Figure 5). The AFP aptamer was added to the screen-printed carbon electrodes (SPCE) surface modified with Au–platinum metallic NPs and RGO–chitosan–ferrocene nanohybrids (Au–Pt NPs/RGO–CS–Fc). This platform was able to quantify a low detection limit of 0.3013 ng/mL within a wide linear range between 0.001 and 10 µg/mL.[85]

Prussian blue (PB) NPs have also gained considerable attention in construction of electrochemical biosensors because of their beneficial properties including easy

Table 4. Electrochemical aptasensors for AFP detection.

Detection methods	Strategy/nanomaterial	Aptamer type	Aptamer sequences	Limit of detection (LOD) (ng/mL)	Linear range (ng/mL)	Recovery rate in human serum samples (%)	References
*Electrochemical impedance spectroscopy (EIS)	Exonuclease-induced target recycling and methylene MB-DNA-AuNP probe	DNA1 DNA2	ACAGCACCACAGACCACGCA CTGGGTTGGGTTGGGTTGGG ATGGTGCTGTG	0.00000027	0.00001–100	–	[69]
EIS	Core-shell guanosine-borate hydrogel-modified Au electrode	–	GTGACGCTCCTAACGCTGACTCAG GTGCAGTTCTCGACTCGGTCTTGA TGTGGGTCCTGTCCGTCCGAACCAATC	0.000076	0.0005–100	91	[70]
EIS	Self-healing G-PyB/KCl hydrogel	–	–	0.51	0.001–0.5	–	[39]
Cyclic voltammetry (CV)	GO//GCE	DNA	GTGACGCTCCTAACGCTGACTCAGG TGCAGTTCTCGACTCGGTCTTGATG TGGGTCCTGTCCGTCCGAACCAATC	0.003	0.01–100	96–102.1	[71]
Alternating current voltammetry (ACV)	MB-DNA-AuNP probe	DNA	GACCCGGGAAGGCAGGAAGACAAACAA GCTTGGCGGCGGGAAGGTGTTAAATTCC CGGGTCTGCGTGGTCTGTGGTGCTGT	0.0000002694	–	–	[69]
Differential pulse voltammetry (DPV)	Modified Au electrode with spindle-shaped Au nanostructure	–	GTGACGCTCCTAACGCTGACTCAGGTGCA GTTCTCGACTCGGTCTTGATGTGGGTCCT GTCCGTCCGAACCAATC	0.00023	0.005–10	–	[72]
DPV	GO nanosheet-modified Au electrode/PBNPs-labeled apt	DNA	GGCAGGAAGACAAACAAGCTTGGCGGCGG GAAGGTGTTTAAATTCCCGGGTCTGCGTGG TCTGTGG TGCTGT	0.0063	0.01–300	–	[73]
DPV	Apt/Peptide/PANI/GCE	–	–	0.00000059	0.000001–1	–	[74]
DPV	TH/RGO/AuNPs	–	GTGACGCTCCTAACGCTGACTCAGGTGC AGTTCTCGACTCGGTCTTGATGTGGGTC CTGTCCGTCCGAACCAATC	50	100–100,000	101.52–107.95	[75]
DPV	Apt/GE/HCR	–	–	0.000041	0.0001–100	103.5–108	[34]
DPV	ConA/AgNPs	DNA	–	0.00876	0.05–10	–	[76]
DPV	Au–PtNPs/RGO–CS–Fc/SPCE	–	TGACGCTCCTAACGCTGACTCAGGTGC AGTTCTCGACTCGGTCTTGATGTGGGT CCTGTCCGTCCGAACCAATC	0.3013	1–10,000	102.3–118.09	[48]
DPV	Mixed self-assembled aptamers and zwitterionic peptides	–	–	0.0000031	0.00001–0.1	–	[77]
DPV EIS	3D nitrogen-doped mesoporous carbon NMs	–	–	0.0000608 0.0000618	0.0001–100	–	[78]
Square wave voltammetry (SWV)	Rod-immobilized DNA-LaMnO3 perovskite-metal ions encoded probes	DNA	–	0.00034	0.0001–100	–	[79]
SWV	PtNPs/GO-COOH/SPGE	–	GTGACGCTCCTAACGCTGACTCAGGTGC AGTTC-TCGACTCGGTCTTGATGTGGGT CCTGTCCGTCCGAACCAATC	1.22	3–30	97–108.9	[80]
	Photoelectrochemical (PEC)	MoS2/Au/GaN	DNA GTGACGCTCCTAACGCTGACTCAGGTG CAGTTCTCGACTCGGTCTTGATGTGGG TCCTGTCCGTCCGAACCAATC	0.3	1–150	85.2–91.7	[81]

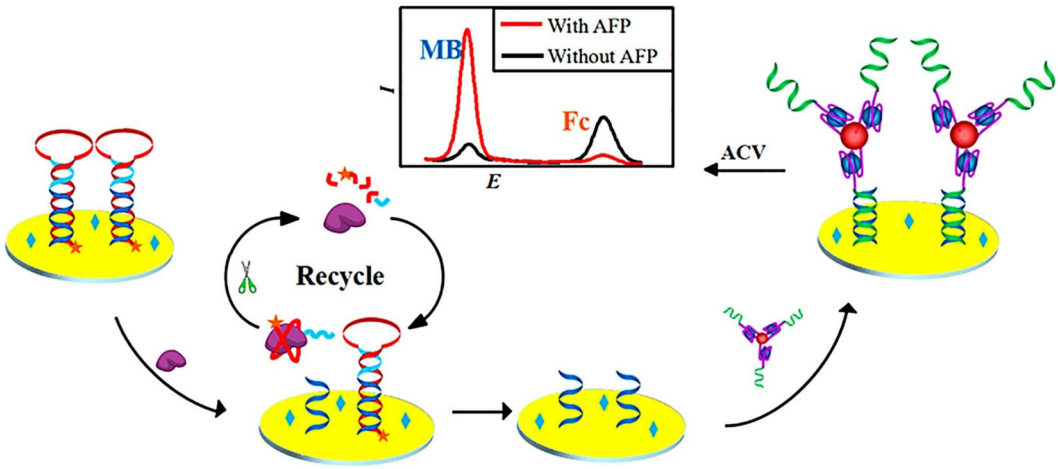

Figure 4. Schematic illustration of bi-directionally amplified ratiometric electrochemical aptasensor for the ultrasensitive detection of AFP. Reprinted with permission from Yang, Zhao, Zhang, Wen, and Zhu.[69]

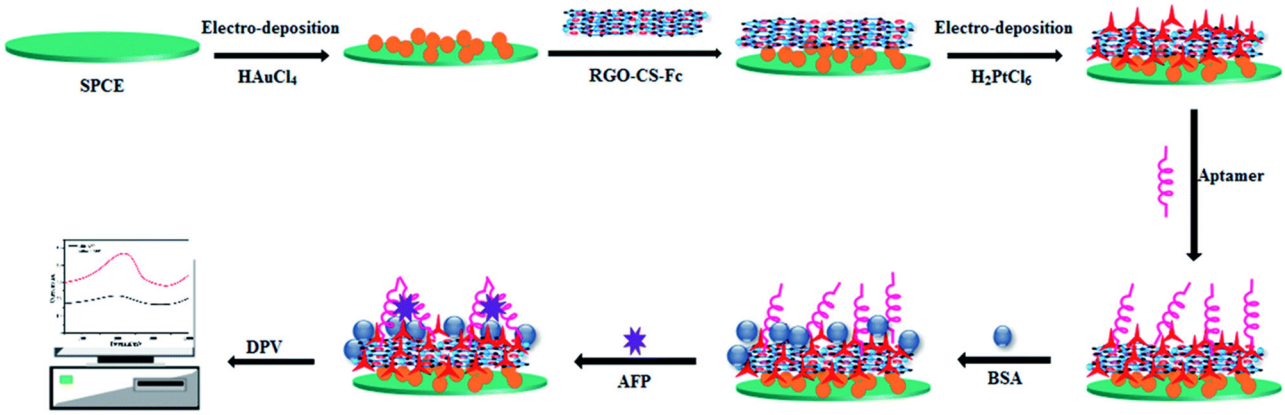

Figure 5. Schematic illustration of AFP electrochemical aptasensor based on Au–Pt NPs/RGO–CS–Fc nanohybrids and AFP aptamer. Reprinted with permission from W. Li et al.[85]

preparation and labeling, as well as great electrochemical signal enhancer.[86,87] Therefore, an enzyme-free electrochemical aptasensor was fabricated for AFP quantification in HCC patients using a GO nanosheet-modified Au electrode and PBNPs as the signal-producer tag, enhancing the target recycling amplification. The aptasensor was developed based on the π–π stacking reaction between the immobilized GO and the PBNP-labeled aptamer. Through forming AFP-aptamer complex, the PBNP was dissociated from the nanosheets and DPV signals were decreased with the increment of the analyte concentration. The range of detection was reported to be from 0.01 to 300 ng/mL and a LOD of 6.3 pg/mL was achieved using this aptasensing platform.[73]

Another sensitive, selective and label-free aptasensor was also constructed by Mohammad Heiat et al. by means of Au electrode modified with spindle-shaped Au nanostructure that improved the area of AFP aptamer immobilization. This aptasensor presented a wide range of linear response from 0.005 to 10 ng/mL and a low LOD of 0.23 pg/mL by employing DPV method. Furthermore, AFP detection using this aptasensor in 20 real clinical serum samples showed that the diagnostic sensitivity and specificity were 85% and 86%, respectively.[72]

In another work conducted by Tao Gao et al, an electrochemical aptasensor was designed using a dual-aptamer hairpin DNA probe for simultaneous detection of AFP and microRNA-16 as two serological biomarkers in HCC diagnosis. The electrochemical signals were produced by methylene blue and concanavalin A (ConA) modified silver NPs (AgNPs) upon targeting microRNA-16 and AFP introduction, respectively. DPV analytical technique was employed for this assay with a linear range of 50 pg/mL to 10 ng/mL for AFP.[76]

Min Cui et al. also designed a selective and sensitive electrochemical aptasensor for AFP assay. Briefly, long-chained aptamers and architectured short-chained zwitterionic peptides, which prevent nonspecific reactions, were attached to the surface of an Au electrode. Addition of AFP led to the conformational changes of bound aptamers and electrochemical signals and DPV method was applied for the monitoring of quantification. The LOD of this system was reported to be down to 3.1 fg/mL in a linear range of 10 fg/mL to 100 pg/mL.[77]

Similarly, Nianzu Liu et al. presented a DPV-based aptasensor for the detection of AFP by employing hierarchically designed zwitterionic peptides immobilized on polymer polyaniline (PANI)/glass carbon electrode (GCE) platform and

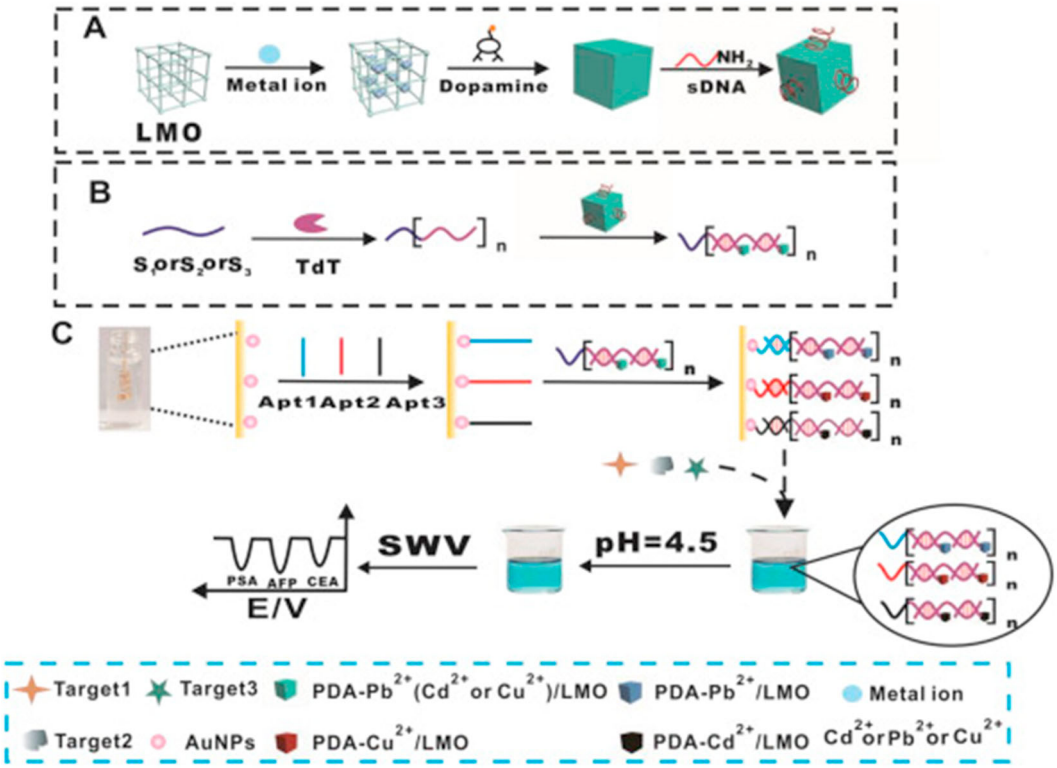

Figure 6. Schematic illustration of AFP electrochemical aptasensor based on the stirring rod-immobilized DNA-LaMnO3 perovskite-metal ions encoded probes. Reprinted with permission from W. Wang, Wang, Xie, Wu, and Gan.[79]

the subsequent modification of the aptamers. Because of the great antifouling properties of these peptides, the aptasensing platform established a superior LOD of 0.59 fg/mL with quantification range from 1 fg/mL to 1 ng/mL.[74]

Bing Han et al.[34] developed a mild reduction-promoted sandwich electrochemical aptasensor for AFP assay. The AFP-specific aptamer was attached to an Au electrode and hybridization chain reaction employed for signal amplification. This platform detected AFP with a LOD of 0.041 pg/mL and linear range of 0.0001 to 100 ng/mL with DPV method.[34]

Wenhai Wang et al. have also recently reported a sensitive and rapid electrochemical aptasensor employed for multiple quantification of tumor markers including AFP, CEA, and PSA based on the competitive target binding with the stirring rod-immobilized encoded probes (DNA-$LaMnO_3$). As indicated in Figure 6, the aptasensor was designed by the immobilization of three hyperbranched DNA on $LaMnO_3$ encapsulating Pb, Cd or Cu ions as electrochemical signal sources. The three aptamers were then attached to the stirring-rod and hybridized with the probes. The sensing platform reached a low LOD of 3.6×10^{-4}, 3.4×10^{-4}, and 2.8×10^{-4} ng/mL for CEA, AFP, and PSA, respectively using SWV technique.[79]

Another novel label-free electrochemical aptasensor was also designed for AFP quantification using PtNPs, as electrical conductivity enhancer on carboxylated GO (GO-COOH) modified screen-printed graphene-carbon electrodes (SPGE) platform. AFP-specific aptamers were also immobilized on the surface of platform as bio-recognition elements. The detection process was performed by SWV technique and showed a good stability and sensitivity in human serum samples. The linear range from 3 to 30 ng/mL with LOD of 1.22 ng/mL.[80]

4.3. Photoelectrochemical AFP aptasensors

Photoelectrochemical (PEC) technique has also the beneficial features of both fluorescence and electrochemical sensing platforms and has been wieldy applied as diagnostic devices.[88]

Recently, Danil Hu et al. introduced a highly sensitive and selective PEC aptasensor for AFP assay using Au/n-type gallium nitride (GaN) photoelectrode. The AFP-specific aptamer with 5′-HS-$(CH_2)_6$-GTG-ACG-CTC-CTA-ACG-CTG-ACT-CAG-GTG-CAGTTC-TCG-ACT-CGG-TCT-TGA-TGT-GGG-TCC-TGTCCG-TCC-GAA-CCA-ATC-3′ sequence was immobilized on the surface of this electrode via Au-S bonds. Upon the addition of AFP, suppression of the sensor photocurrent was decreased through blockage of charge carriers transfer of GaN to MoS2. LOD in this aptasensor was determined 0.3 ng/mL.[81]

4.4. Brief overview of electrochemical AFP aptasensors

Overall, between various electrochemical biosensing systems including EIS, voltammetry and PEC, the DPV was the most extensively used technique for highly sensitive and selective AFP quantification and the ACV based aptasensing platform exhibited the lowest LOD (0.0000002694 ng/mL) for AFP assay.

Table 5. Comparison of analytical performance of various AFP commercial detection kits.

Commercial AFP kits	Assay sensitivity (ng/ml)	Assay range (ng/ml)	Sample types	Detection methods	Assay time
LifeSpan BioSciences	0.259	0.625 – 40	Plasma, serum, tissue homogenates	Colorimetric	–
RayBiotech	0.006	0.006 – 1.8	Cell culture supernatants, plasma, serum	Colorimetric	–
Wuhan Fine Biotech Co., Ltd.	0.188	0.313–20	Serum, plasma and other biological fluids	–	–
Antibodies-online	0.26	0.078–50	Cell culture supernatant, cell lysate, plasma, serum, tissue homogenates	–	3 h
Ansh Labs	0.074	6, 5–500	Serum	–	1 h
Eagle Bioscience	0.35	5–200	Plasma, serum	Colorimetric	1.5 h
R&D Systems	0.046	0.31–20	Cell culture supernatants, serum, plasma	–	4.5 h
Biorbyt	1.332	1.563–100	Serum, plasma, cell culture supernatants, tissue homogenates	–	–
Thermo Fisher Scientific	–	0.00737–1.800	Plasma, serum, Supernatant	–	–
Aviva Systems Biology	<0.156	–	Serum, plasma, tissue homogenate, cell culture supernatant, other biological fluids	Colorimetric	3 h

5. Comparison of the performance of AFP aptasensors

As summarized in Tables 3 and 4, several optical and electrochemical aptasensing platforms with different detection means and NMs have designed for AFP determination. Most of the reported aptasensors exhibited high sensitivity and selectivity, accurate outcomes and repeatability for AFP assay. Real-time, quantitative and direct detection are several advantages for optical aptasensors and also no warm-up time is needed. However, their construction is time-consuming and has low portability. Besides, application of fluorescence-based techniques has been limited due to their disability in multiple biomarker detection that is of high importance in clinical diagnostic tools.[2,89,90] Electrochemical aptasensors have also beneficial properties including quick, simple, low-cost, ultrasensitive, quantitative and robust AFP detection. Moreover, these systems are very appropriate for point-of-care diagnostic methods and low sample volume is required. Nonetheless, false positive outcomes and restricted control on the working electrode at higher currents are some disadvantages of these methods.[2,14,27]

Additionally, DNA aptamers have been mostly employed for construction of these platforms (Tables 3 and 4) due to their greater stability, cost-effectiveness, and easy functionalization as compared with RNA aptamers.[91] The DNA AFP-specific aptamer with a sequence of 5′-GTGACGCTCCTAACGCTGACTCAGGTGCAGTTCTCG-ACTCGGTCTTGATGTGGGTCCTGTCCGTCCGAACCAA-TC-3′ was the most common aptamer employed for fabrication of the aptasensors.

Moreover, the length of the aptamer, type of NMs, further signal amplification approaches can modify the performance of these aptasensing platforms.

6. Comparison of AFP commercial detection kits with the established aptasensors

Presently, antibody-based colorimetric detection methods such as ELISA are the most frequent commercial kits for AFP quantification as summarized in Table 5. As compared with aptasensors, these methods need a larger sample size, complicated devices and procedures, expensive antibodies, as well as long incubation times.[2,14] Besides, antibody-target variable affinity, and lower sensitivity and limited LOD are other restrictions of the existing kits.[92] As indicated in Table 5, RayBiotech is a commercial ELISA kit for human AFP detection which has shown a great sensitivity with a LOD of 6 pg/mL, though, the established aptasensing platforms are capable of detecting AFP even in the range of 0.0002694 pg/mL. Since quantification of AFP trace amounts is of highly rewarded for rapid diagnosis of HCC or monitoring of cancer therapy, the various optical and electrochemical aptasensors or nano-aptasensors could have higher sensitivity than the commercial detection kits. Besides, as compared with antibody-based colorimetric detection methods, these aptasensing platforms can be innovative emerging candidates for point-of-care analysis in clinical diagnostic tools which is currently at the nascent phase.

7. Conclusion and future perspectives

In recent years, noticeable progress has been made in fabrication of optical and electrochemical aptasensing platforms for AFP analysis as the leading biomarker for rapid diagnosis of HCC. Aptamers pose superior properties as compared with antibodies such as great sensitivity, stability, low-costs, and easy production which have made them applicable options for designing AFP aptasensing platforms. Researchers have further developed this field to fabricate a number of novel signal transduction schemes and enhance sensitivity and performance of the aptasensors. Although these platforms have achieved some success and are applicable in real clinical samples, they have not been established as routine clinical diagnostic devices in laboratories. In fact, there are still several challenges requiring to be addressed. For example, sample conditions including viscosity, ionic strength, and aptamer nonspecific interactions with the sample matrix need to be more noticed. Although electrochemical aptamer-based biosensors have achieved lots of beneficial features such as multiplexed analysis, rapid detection, high sensitivity and specificity, their stability should be

enhanced and the strict pretreatments of samples are necessary. Additionally, fluorescent aptasensors are potent in highly sensitive, efficient, simple and rapid target detection. However, fluorescence background and lifetime affect the stability and accuracy of the designed aptasensing platforms. Chemiluminescent aptasensors also have multifaceted processes and require enzymes and special devices. Furthermore, SERS-based aptasensors have not shown good reproducibility yet that should be enhanced in the future. Furthermore, more effort is essential for establishment of convenient AFP aptasensing platforms for point-of care diagnosis.

Application of NMs in designing aptasensors have also mainly enhanced the AFP limit of quantification in the ranges of fg/ml or even ag/ml that offers novel promising approaches for early diagnosis of HCC. However, the risk factors caused by NMs are one of the greatest concern that requires great notice for in vivo AFP assay. In addition to the restrictions regarding their intrinsic features, the difficulty in bio-conjugation chemistry can lead to the development of ineffective aptasensors. In this order, improvement of existing methods and searching for novel high performance NMs is beneficial.

Aptasensors mostly meet point-of-care diagnostic necessities. The aptamers incorporation into microfluidics sensing platforms, paper-based analytical tools, wearable electrochemical sensors, disposal electrochemical sensors, smart phone-based electrochemical sensors and lab-on-a chip areas for point-of-care diagnosis have also recently gained notable attention because of their high sensitivity, simplicity, miniaturization, and low costs as well as noninvasive analytical ability and rapid and real-time monitoring.

As a consequence, given the recent progress in aptasensors, a wide range of developments probably will occur in the development of efficient, small and cost-effective AFP aptasensors in the future in parallel with lab-on-a chip tools and miniaturized platforms with potentially considerable use in clinical diagnostics.

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Conflict of interest

Authors declare that they have no conflict of interest.

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