Photoelectrochemical aptasensors for detection of viruses

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Abstract
Photoelectrochemistry (PEC) is a dynamic discipline studying the effect of light on photoelectrode or photosensitive material, and the conversion from solar energy into electrical power. The basic PEC process refers to the oxidation or reduction reactions between electrochemical active species in solution and photoactive materials that occurred at the electrode/electrolyte interface during illumination. In recent years, the PEC biosensing approaches have also been developed by the combination of the PEC technique with bioanalysis, where the interaction between biological recognition element and analyte influences a photocurrent signal. This involves the charge and energy transfer of PEC reaction between electron donor/acceptor and photoactive material upon light irradiation. Coupling the advantages of PEC bioanalysis and aptamers has provided new concepts for highly selective and sensitive biosensors development, applicable in human health monitoring and environmental protection. In a typical assay, a photoactive material converts the affinity binding properties of aptamers into a detectable electrical signal, presenting an innovative method for probing numerous aptamer–analyte interactions. Using different aptamer probes aiming for specific purposes, more sensing strategies with rational design and exquisite signaling mechanisms have been proposed. This review concentrated on the current topic of PEC aptasensors that are used for the detection of viruses. The prospects in this area are also discussed.

Keywords Photoelectrochemistry · Aptamers · Biosensor · Viruses · Photocurrent · Impedance

Introduction
Conventional and advanced methods used to detect viruses in clinical diagnostics include electron microscopy, enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), next-generation sequencing, and mass spectrometry. Although these methods provide correct and accurate detection of viruses/viral infection-related species, each suffers from characteristic disadvantages, which can be summarized as (i) false positive or false negative results due to interferences, (ii) high cost of reagents and equipment, (iii) risk of contamination during sample preparation, (iv) long evaluation times, (v) the need for qualified personnel and their deployment on a massive scale. This all means considerable complications in contrast to simple devices or tests [1, 2]. Due to the disadvantages of conventional methods, researchers have sought other alternatives for detecting viral pathogens.
In recent years in vitro diagnostic technologies have taken over a new dimension with the arrival of point-of-care (POC) testing which has created a paradigm shift in the field of diagnostics and biosensing by enhancing the speed and accuracy of diagnoses. Furthermore, a reduction in analysis time, cost, dependence on sophisticated equipment and skilled personnel makes POC testing the preferred choice [3]. Especially now, there is an urgent need for the development of such rapid, selective, sensitive diagnostic systems to achieve the mass and targeted SARS-CoV-2 detection, therefore, to manage the COVID-19 pandemic through the understanding of infection progression and appropriate therapy decisions [4].

Lately, aptamer-based biosensors as ultrasensitive and accurate assays at the POC are being developed. Aptamers are single-stranded synthetic nucleic acid-based molecules (more recently, peptides) shorter than 100 nucleotides in length, but with properties comparable to those of antibodies [5]. Because of their great affinity, specificity, and stability, aptamers are of special interest in different uses from chemical biology to medicine. Especially fascinating is their application in biotechnology and diseases treatment [6]. These artificial sequences can fold into complex secondary and tertiary structures, forming binding pockets and clefts with unique and extremely high affinity and specificity for a large variety of target molecules, such as metal ions, drugs, peptides, proteins, and even the entire viruses or cells [7]. Although the development of antiviral aptamers is not an easy task, aptamers that recognize viral proteins have already been successfully implemented in the rapid detection of viruses and antiviral agents [8, 9], such as SARS [10], avian influenza virus (H5N1) [11], zika [12], and recently SARS-CoV-2 [13]. This was mainly possible due to the fact, that the advanced SELEX (systematic evolution of ligands by exponential enrichment) method (Viro-SELEX) suitable for the development of membrane proteins aptamers was developed [14].

Aptamer-based biosensors, also called aptasensors, use aptamer as bioreceptor (also named capturing aptamer/probe) or transducer (also named signal aptamer/probe). Aptasensors are mainly classified into optical and electronic aptasensors based on the type of transducer [15]. Recently, photoelectrochemical (PEC) aptasensing has become an important method for bioanalysis due to the target-dependent binding with high specificity and affinity, as confirmed by the increasing number of papers published on the subject (Fig. 1). In a typical configuration, aptamers are immobilized onto the transducer surface for the subsequent target recognition and binding, which is recorded by the transducer as the variation of the electrical signal [16]. In PEC detection, light is used as an excitation source, while current is measured as the output detection signal. The ability to couple the photoexcitation process with electrochemical detection makes PEC sensors the unique advantage of being both optical and electrochemical sensors [17]. PEC analysis presents the advantages of high sensitivity, desirable selectivity, low background signal, simple equipment, and easy miniaturization, obtaining great progress in sensing applications [18, 19]. The growing interest in PEC bioanalysis [20] has resulted in essential progress in its analytical performance and bio-detection applications, including immuno-sensing [21], enzymatic biosensing [22], DNA analysis [23], RNA analysis [24, 25], and of course aptasensing [26].

Using illustrative examples, this review provides the introductory concept, bioanalysis development, signaling strategies, and the state of the art in this field of PEC aptasensors for the detection of viruses. As we are sure, many research papers are dealing separately with topics of PEC bioanalysis (or electrochemical analysis) and aptamer-based sensors applicable for the detection of viruses, this review will provide insight into the highly specific area of PEC aptasensors and the use of photosensitive materials for biosensors development.

**Photoactive materials used at PEC aptasensors**

To date, as an important PEC active material, quantum dots (QDs) have attracted much attention due to their unique size- and shape-dependent properties. When QDs are illuminated, an electron–hole pair is generated in the conduction and valence band. Electrons transfer from the conduction band of the QDs to the electrode with an appropriate energy level and the holes react with the electrolyte at the semiconductor surface, resulting in photocurrent. To enhance the PEC signals, the method of coupling narrow bandgap
semiconductors with wide bandgap ones, which can increase the charge separation efficiency, has wake more attention. For example, cadmium sulfide (CdS) and cadmium telluride (CdTe) are well-known narrow direct bandgap semiconductors and are widely used for sensitizing TiO₂, ZnO, or WO₃ [27].

Although aqueous QDs have captured wide attention in PEC sensor applications, the charge detachment and recombination take place in an ultrafast time, resulting in poor photoconversion efficiency. To address these issues, many carbon-based nanomaterials, such as carbon nanotubes and graphene, have been introduced in PEC sensors construction for their large specific surface areas, high electrical conductivity, and chemical stability, which could improve the electron transfer efficiency [28, 29]. Recently, graphitic polymeric carbon nitride (g-C₃N₄), which is composed of carbon, nitrogen, and some minor hydrogen content has attracted great interest benefiting from its cheap availability, high thermal and chemical stability, and good visible light response. However, the pure g-C₃N₄ is still limited in practical application because of the low conversion efficiency caused by the high recombination rate of photogenerated charges. Therefore, coupling g-C₃N₄ with well-matched band structure semiconductors can effectively promote photoelectric conversion efficiency [30].

Besides mentioned above, a valid and practicable way to boost PEC performance is by doping other photoactive materials with noble metals. Among them, photoactive materials doped by gold nanoparticles (AuNPs) play a tremendous role in PEC aptasensors application. They have excellent photoelectric properties, electrical conductivity, plasma resonance, catalytic activity, size and shape controllability, and biocompatibility [31]. The Fermi energy of AuNPs and the charge transfer efficiency of the AuNPs/semiconductor composites can be controlled by changing the size of the AuNPs. Moreover, the surface of AuNPs can be easily modified, and the aptamers can be bonded to the surface of AuNPs via the Au–S bonds, which are beneficial to the surface functionalization and the fabrication of sensors [32].

Among these, other well-known semiconductor materials could be also used for aptasensors construction. Recently, the interest in polymer-based photoactive materials has also emerged as they are efficient and less toxic alternatives to certain kinds of inorganic semiconductors and sensitizers [19]. However, until now, they have not been reported for the PEC detection of viruses.

**Detection principles**

The fabrication of the PEC aptasensor is monitored by changes of the photocurrent in a solution usually containing ascorbic acid as an electron donor. After the photoactive materials (or nanocomposites) are modified on the bare electrode surface, an increased photocurrent is observed, compared to the bare electrode. This can be attributed to the generation of electron donor molecules by the photoactive material, which could capture the photogenerated holes and improve the photocurrent response [33]. The final detection strategy of the virus could vary, depending on the mobility of aptamer, whether it is present in solution, thus, can undergo the hybridization mechanism with the surface-immobilized capturing probe (Fig. 2a) or it is covalently fixed to the electrode surface (Fig. 2b). After the immobilization of the capturing probes or aptamers, the PEC signal decreases due to the diffusion limitation of the electron donor molecule to the electrode surface coated with photoactive materials. As the target aptamer hybridizes with the immobilized probe on

![Fig. 2 Principle of the PEC sensor with a signal-on and b signal-off strategies for the detection of viruses](image-url)
the surface of the electrode, the PEC signal decreases even more. This could be explained as a hindrance of the diffusion of electron donor to the electrode surface by target aptamer. Upon adding the target, the prehybridized aptamer binds to the target while leaving the electrode surface, which leads to a signal increase (Fig. 2a). Otherwise, the immobilized aptamer binds the target by changing its conformation which causes the signal to decrease because of the steric hindrance (Fig. 2b).

Electrochemical impedance spectroscopy (EIS) is an effective and sensitive method to obtain detailed information on the modified electrode surface. Usually, the Nyquist plots of the impedance spectrum corresponding to the different stages of sensors modification are shown and compared. The EIS of the bare electrode usually shows a straight line with a very small semicircle, indicating that the used electron donor could reach the electrode surface and exchange charge conveniently [34]. After the modification with the selected photoactive materials, the charge transfer resistance significantly increases, indicating that the photoactive materials are successfully immobilized on the electrode surface and decrease the electron transfer efficiency. When the modified electrode is assembled with probe aptamer sequence and later with target aptamer sequence, the charge transfer resistance increases progressively, indicating that the electron transfer was obstructed by the assembled DNA strands.

Applications of PEC aptasensors in viruses’ detection

A highly effective way to improve the prognosis of viral diseases and to determine the outcome of infection is an early, fast, simple, and highly selective, and sensitive diagnosis of viral pathogens in biological fluids [35]. Following, we discuss in detail some detection approaches of three different viruses using PEC aptasensors, as promising analytical tools for the application in real biological samples. The results are summarized in Table 1.

Table 1 PEC aptamer-based biosensors for the detection of viruses

| Virus | Sensor | Detection technique | Matrix | Linear range (fM–nM) | LOD/fM | RSD/% | References |
|-------|--------|---------------------|--------|----------------------|--------|-------|------------|
| HIV-1 | ALP-AuNPs-DNA/H-DNA/QDs2/QDs1/ZnO/Au-PWE | Photocurrent EIS | serum | 1 fM–1 nM | 0.65 | 4.7 | [38] |
| HTLV-II | pDNA/ZnO/GR-CdS:Mn/ITO | Photocurrent EIS | – | 0.1–0.5 fM | 0.033 | 5.3 | [41] |
| SARS-CoV-2 | Aptamer/AuNPs/Yb-TCCP-4/GCE | Photocurrent EIS | pharynx swabs | 3.7–59 fM | 0.53 | 2.8 | [45] |
| SARS-CoV-2 | Aptamer/Chitosan/CdS QDs-g-C3N4/ITO | Photocurrent EIS | saliva | 0.5–32 nM | 1.20 | 5.8 | [46] |

Abbreviations: ALP, alkaline phosphatase; AuNPs, gold nanoparticles; CdS QDs, cadmium sulfide quantum dots; EIS, electrochemical impedance spectroscopy; g-C3N4, graphitic carbon nitride; GCE, glassy carbon electrode; GR, graphene; H-DNA, hairpin structure DNA; HTLV-II, human T-cell lymphotropic virus type II; ITO, indium tin oxide; pDNA, probe DNA; PWE, paper working electrode; QDs, quantum dots; Yb-TCCP, ytterbium and tetrakis(4-carboxyphenyl)porphyrin junction.
Fig. 3  Examples of the PEC aptasensors design for the detection of viruses. a Fabrication process of the PEC biosensor for detection of HIV-1. Reprinted with permission from Ref. [38]; b schematic illustration of the fabrication process of the PEC aptasensor for the HTLV-II detection. Reprinted with permission from Ref. [41]; c mechanism of plasmon-enhanced PEC sensing at SARS-CoV-2 spike glycoprotein detection. Reprinted with permission from Ref. [45].
biosensor had excellent feasibility, even in human serum samples, which showed great potential in practical applications. The stepwise assembly processes of the PEC biosensor fabrication were also characterized by the EIS technique.

**Human T-cell lymphotropic virus type II**

Human T-cell lymphotropic virus type II (HTLV-II) is a human retrovirus that is a causative agent of adult T-cell leukemia and lymphoma, which is associated with cancers, pulmonary and urinary tract symptoms, as well as increased mortality. HTLV-II DNA can be found at trace level in clinical samples, hence, an effective method for sensitive detection of this retroviral DNA is necessary. Therefore, the development of nucleic acid-based techniques which can trace the presence of specific DNA sequences has aroused researchers’ interest [39, 40].

A “signal-on” PEC aptasensor based on dual signal amplification strategy for the detection of HTLV-II DNA (target DNA) was developed by Shen and co-workers [41], using graphene–CdS:Mn/ZnS nanocomposites as photovoltaic material. The photocurrent intensity of the graphene–CdS:Mn nanocomposites was the strongest, because the doped Mn ions could trap electrons. Furthermore, the introduction of graphene could simplify the separation of charge carrier, thus, result in enhanced photocurrent intensity. As a wide bandgap semiconductor, ZnS could be used to reduce the number of surface defects of the CdS nanoparticles and to avoid the recombination of charge carriers. When the electrodes prepared in the described way were finally coated with two layers of ZnS, the corresponding photocurrent achieved a maximum value. The photocurrent decreased with the assembly of the probe DNA sequence, which may be attributed to the increased steric hindrance of the immobilized aptamer. As shown in Fig. 3b, after target DNA hybridized with probe DNA on the electrode surface, biotin was incorporated to reduce the unspecific reaction. Furthermore, biotin could conjugate with avidin (form avidin–alkaline phosphatase, which was used to amplify the sensitivity), and the photocurrent response could be greatly enhanced. The results were confirmed by EIS measurements as well.

**Severe acute respiratory syndrome coronavirus 2**

Since late December 2019, the coronavirus pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been surging rapidly around the globe. Therefore, highly sensitive and specific diagnostic methods that can distinguish infected from healthy or other virus-infected individuals are essential. Currently, various methods for the clinical diagnosis of COVID-19 have been reported [42]. Electrochemical biosensors and especially aptasensors represent an important role in the development of POC devices for the early and sensitive detection of SARS-CoV-2 [8, 13, 43, 44].

A two-dimensional metal–organic framework-based PEC aptasensor with high sensitivity and stability for SARS-CoV-2 spike glycoprotein (S protein) detection was developed by Jiang and co-workers [45] (Fig. 3c). A 2D metal–organic framework-based Schottky junction, namely, AuNPs/Yb-TCPP (ytterbium and tetrakis(4-carboxyphenyl)porphyrin junction), was successfully fabricated by a simple in situ synthesis strategy. This junction greatly improved the electron–hole separation efficiency of 2D Yb-TCPP nanosheets at the glassy carbon electrode (GCE). The enhanced electron–hole separation effect was verified by EIS. As expected, with the increase AuNPs loading on the surface of 2D Yb-TCPP nanosheets, the radius of the Nyquist curve decreased gradually, indicating that the charge transfer resistance decreased gradually, which could accelerate the photoelectron transfer and increase the photocurrent. The S protein could be captured by the modified aptamer on the surface of AuNPs/Yb-TCPP/GCE. With the probe aptamer assembled on the AuNPs/Yb-TCPP-4/GCE, charge transfer resistance increased. After blocking the nonspecific sites by bovine serum albumin, the charge transfer resistance value was further increased due to its high steric hindrance and low conductivity. Subsequently, after incubation with S protein, the charge transfer resistance value increases sharply due to the steric hindrance of S protein, manifesting that the S protein was successfully captured on the electrode surface by its specific aptamer. Due to the low conductivity and large steric hindrance, the electrons transfer between the electrode and electrolyte was blocked, leading to the decrease of the photocurrent. Based on the photocurrent change against S protein concentration, a sensitive PEC aptasensor was developed to detect S protein, which can be considered as a useful supplement for nucleic acid detection, and even provide a potential strategy for direct detection of SARS-CoV-2.

A PEC aptasensor for the determination of the SARS-CoV-2 receptor-binding domain was developed by Tabrizi and co-workers [46], using graphitic carbon nitride–cadmium sulfide quantum dots nanocomposite as photocative material. Here similarly, after the modification of the indium tin oxide electrode with Chitosan/CdSQDs-g-C$_3$N$_4$, the recorded PEC signal dramatically increased compared to the unmodified electrode. After the immobilization of the aptamer probes, the PEC signal decreased due to the diffusion limitation of the electron donor molecule to the electrode surface coated with a photocative nanocomposite. As SARS-CoV-2 receptor-binding domain interacted with the immobilized aptamer on the surface of the electrode, the PEC signal decreased even more due to the hindered...
diffusion of electron donor to the electrode surface. The applicability of the proposed sensor was also studied in human saliva samples.

From the mentioned above, it is clear, that the field of PEC aptasensing of viruses is still relatively new and not commonly used, although photoelectrochemistry and electrochemical aptasensors themselves are currently “gaining momentum”. Despite several advantages mentioned above, PEC aptasensors have their pitfalls which include problematic multiple analyte detection, cross-reactivity among some molecules leading to false results, and laborious immobilization procedures leading to loss of precision. On the other hand, the published papers showed possible applications of highly sensitive and selective biosensors even for real samples analysis, which gives a promise for the implementation of these sensors in POC testing.

Conclusions

Numerous biosensors have been developed over the past decades for specific detection of biomolecules, to meet the increasing demands for early medical diagnostics and point-of-care treatment of emerging diseases. The fusion of photoelectrochemical (PEC) bioanalysis and aptamers have offered great advances and novel developments in PEC aptasensing in recent years, using obvious advantages in terms of high sensitivity and selectivity of PEC bioanalysis and the affinity of the aptamers. The present review addresses the basic concepts of PEC aptasensing of viruses, which has become an important issue due to the recent pandemic situation. Aptamer-based biosensors based on PEC detection principles for viral detection can be developed relatively fast to discriminate between closely related molecules. This fact makes them ideal for developing the point-of-care diagnostics against rapidly emerging threats.

Similarly, other nucleic acid-based antiviral compounds such as deoxyribozymes (DNAzymes) have been developed to demonstrate potential applications in the management of several respiratory virus infections. DNAzymes are G-rich single-stranded DNA sequences that can fold into four-stranded structures known as G-quadruplexes. Some G-quadruplexes have proven to bind hemin and mimic the formation of a catalytic complex with the peroxidase activity [47]. DNAzyme and aptamer conjugations have already been exploited for sensitive and accurate detection of several target molecules, such as ATP, cocaine, adenosine, thrombin, and mycotoxins [48]. The specific binding between aptamer and DNAzyme would influence the catalytic activity of DNAzymes to produce a quantifiable signal. Maintaining or enhancing the peroxidase activity of DNAzymes during conjugation can lead to superior biosensing efficiency. Therefore, the development of new functional DNA molecules using the combination of aptamers and DNAzymes as functional nucleic acids could introduce valuable applications in biosensor development [49]. The aptamer/DNAzyme-based photoelectrochemical strategies were also introduced as the new concept of sensing devices and could expand the application range of photoelectrochemical aptasensors in the analytical field [50, 51].

Nanomaterial-based artificial enzymes (nanozymes) have attracted great attention in the past few years owing to their capability not only to mimic functionality but also to overcome the inherent drawbacks of natural enzymes. The catalytic activity of nanozymes, primarily peroxidase-like activity has been utilized extensively for various biosensing applications in combination with modern biological recognition elements, such as aptamers [52]. The nanozymes-based aptasensors are compatible with the existing as well newly developed sensing platforms ranging from colorimetric to electrochemical assays. Moreover, PEC aptasensors using nanozymes as photoactive materials have been recently developed for the ultrasensitive detection of antibiotics in real samples of urine, river water, and milk [53]. Therefore, it seems that the bioconjugation (e.g., a covalent linkage or high-affinity biotin/avidin interactions) or physisorption between aptamers and nanozymes could be achieved through simple and elementary pathway resulting in total cost reduction of the fabrication process and could be considered as affordable and ultrasensitive biosensing platforms for future applications in real samples [54, 55].

Therefore, the coupling of newly developed PEC active materials with high-affinity recognition elements would open new areas in PEC bioanalysis research and extend the PEC biosensors’ applications.

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