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Review

Label-based and label-free electrochemical DNA biosensors for the detection of viruses: A review

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ABSTRACT

Nowadays, the rapid determination of several viruses is highly important. Most of the rapid detection of human pathogen viruses has been developed by using biosensor technology. The detection layer of the biosensor consists of short single-stranded DNA (probe) able to form a duplex with a complementary target nucleic acid fragment with high efficiency and specificity. The probe is associated with a transducer that translates the hybridization event into a physically measurable value based on electrochemical methods. Electrochemical DNA biosensors offer merits such as rapid response, portability, high sensitivity, ease of use, and low detection limit. This review provides an overview of label-based and label-free electrochemical DNA biosensors for the detection of viruses as well as their application in the past four years.

KEYWORDS: electrochemical DNA biosensor, virus detection, DNA hybridization biosensors.

INTRODUCTION

Viral infections are a major threat to public health as well as the global economy. Viruses are pathogens with a diameter of 20-300 nm and a length of 200-1,000 nm that can damage cells, tissues, organs and can cause death in living things. They spread rapidly, mostly through contaminated water, food and body fluids and result in the death of humans and animals worldwide. Thus, their rapid and accurate detection can mean the difference between life and death during viral infections [1].

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DNA is the carrier of genetic information and the base material of biological heredity. Nucleic-acid sequences, which are different in every living organism, virus, or pathogen, provide practical ways to recognize and discern diverse diseases [2]. PCR is the most commonly used DNA identification method, however, the method is often not suitable for rapid on-site analysis as it requires virus isolation and biocontainment, and analysis is time-consuming, needing expensive laboratory tools that are difficult to transport [3]. DNA biosensors offer considerable promise for obtaining the sequence-specific information in human, viral, and bacterial nucleic acid in a portable, faster, simpler manner, and the simplicity of the tool makes it easy to use as compared to conventional assays [4].

A typical biosensor combines a bioreceptor with a transducer. Electrochemical transduction in genosensing facilitates sequence-specific interrogation of target DNA sequences [4]. Electrochemical biosensors have attracted attention because of their advantages such as portability, simplicity of the tool, ease of using, cost-effectiveness, fast response, high sensitivity, and high selectivity [3, 5, 6].

The principle of DNA analysis using electrochemical biosensors is based on the hybridization of the DNA probe sequence with its complementary strand on the electrode surface. The occurrence of specific hybridization was detected using an electrochemical transducer. An electrical signal is produced when target DNA binds to the complementary sequence of the capture or probe DNA in a process called hybridization [6]. The major advantages of electrochemical DNA biosensors compared to other DNA sensors are as follows: amenable to

miniaturization, compatible with microfabrication technique, require simple instrumentation, provide a remarkable sensitivity and selectivity, process rapid response, easy to operate and have high portability, minimum power requirements and low-cost production [7].

A few approaches in the measurement of electrochemical DNA signal have been used, based on measuring electrode potential or voltage difference, measuring current (amperometric), measuring resistance (impedimetry), measuring current with a determined potential range (voltammetry) [3]. Electrochemical detection of DNA hybridization usually involves monitoring the current at a constant potential. DNA hybridization detection methods can be classified into label-free and label-based methods. Label-based electrochemical DNA biosensors use redox-active molecules as indicators to promote electron transfer between the electro-active base and the electrode surface. DNA is detected indirectly by measuring the electrical signals generated by the modified substances. As for the label-free electrochemical DNA biosensors, the structure and composition of the DNA itself are very advantageous for electrochemical detection. Guanine is a nitrogenous base that is more easily oxidized because it has a lower standard reduction potential value than other nitrogenous bases, hence electron transfer can be performed directly on several electrode surfaces [7].

Some of the concerns for developing electrochemical DNA biosensors are probe immobilization capability and working electrode sensitivity. Therefore, to improve the performance of the working electrode, modifications are made with nano-based materials such as gold [8]. This review reports on DNA biosensors, electrochemical DNA biosensors, DNA hybridization in biosensors, and the application of electrochemical DNA biosensors for virus detection.

DNA biosensor

Biosensors are chemical sensors in which the recognition system utilizes a biochemical reaction mechanism [9]. Biosensors were developed in the 1960s by pioneers Clark and Lyons [10]. The biosensor consists of two main components that are connected sequentially, namely a bioreceptor (biorecognition or identification compound), which will recognize the target analyte, and a physical transducer. The biosensor produces an electrical signal that is proportional to the analyte concentration. Most of the biosensors that are often used to detect nucleic acids are electrochemical biosensors [11].

In nucleic acid-based biosensors, the sensing element is an oligonucleotide, with a known base sequence, or DNA or RNA. Nucleic acid biosensors are based on highly specific hybridization of complementary strands of DNA or RNA molecules and play the role of highly specific receptors of biochemical or chemical species [5, 12]. Nucleic acid biosensors are of particular interest because of their ability to obtain sequence-specification information in a faster and simpler manner than traditional methods. Unlike enzymes or antibodies, nucleic acids can be easily synthesized and regenerated for various uses. Nucleic acid biosensors can be more sensitive and specific when combined with polymerase chain reaction (PCR) methods [13].

Electrochemical DNA biosensor

Electrochemical biosensors have been widely used to detect several biomolecules, such as viruses [14], and have attracted attention because of their advantages such as portability simplicity, cost-effectiveness, fast response, high sensitivity, and high selectivity [5, 8, 12].

The basic principle of the electrochemical DNA biosensor is the detection layer of the biosensor consists of short single-stranded DNA (ssDNA probe) able to form a duplex with the complementary target nucleic acid fragment with high efficiency and specificity. The probe is associated with a transducer translating a hybridization event into a physically measurable value [5, 13]. The hybridization process will occur when the target DNA sequence is complementary to the probe DNA immobilized on the electrode surface. If the complementary DNA contains a nucleotide sequence that matches the probe DNA, DNA hybridization will occur to form dsDNA on the electrode surface. When the target sequence pairs correctly with the probe, the hybrid formed on the electrode surface is transformed into an analytical signal *via* a transducer [11].

The properties of working electrode in terms of material type, surface modification and surface area

can strongly influence the DNA hybridization efficiency on the working electrode surface [14, 15]. Further modification using carbon or metallic nanostructures improves sensitivity by enhancing immobilization of recognition elements, or binding of target molecules due to increase in the surface area [8, 9]. The conductivity and chemical processes are important for the performance of the electrode. Carbon (eg. graphite), silicon, platinum, and gold-based electrodes are used depending on the analyte [14].

Voltammetry has grown rapidly to become one of the most widely used electrochemical analysis techniques. In voltammetry, current is measured as a function of changing potential. This current flows from auxiliary electrode to the working electrode, where reduction of the solvent or other components of the solution matrix occurs. Peak current intensity is proportional to the target concentration. Among the voltammetry techniques, cyclic voltammetry (CV), square wave voltammetry (SWV), and differential pulse voltammetry (DPV) are the most frequently used in the development of biosensors [3]. CV is often used for the electrochemical coupling process on the electrode surface. SWV, as a kind of frequency-dependent, versatile, and highly sensitive electrochemical analysis technique, is widely used in quantitative analysis and kinetic studies of materials. DPV possesses the merits of lower background current and higher sensitivity; almost all electrochemical DNA biosensors regard DPV as an analysis technique for determining target concentration. Another electrochemical technique generally applied to electrochemical DNA biosensors is electrochemical impedance spectroscopy (EIS). In this technique, the impedance value is affected by changes in the electric field caused by the interaction between the bioreceptor and the target [4].

DNA immobilization strategies and mechanism of electrochemical detection are two of the most important aspects that should be considered before developing a highly selective and sensitive electrochemical DNA sensor [12]. Some recent strategies for DNA probe immobilization on the surface of electrochemical transducers such as adsorption, covalent bonding, and avidin-biotin interaction on the surface can be carried out by covalent or non-covalent binding on the electrode surface for specific interaction with its complementary DNA target [15, 16] as shown in Table 1.

DNA hybridization in biosensor

Complementary DNA pairing is the basis for the biological recognition process in hybridization biosensors [13]. The hybridization process will occur when the target DNA sequence is complementary to the probe DNA immobilized on the electrode surface. If the complementary DNA contains a nucleotide sequence that matches the probe DNA, DNA hybridization occurs to form dsDNA on the electrode surface [12].

Table 1. Common methods of DNA immobilization.

Methods	Principle	Evaluation
Adsorption	DNA probes are immobilized on the working electrode surface *via* electrostatic adsorption between a negatively charged phosphate group of DNA on the positive charged modified electrodes [15, 16].	Simple, with no need of any chemical reagents and DNA probe modification. Low DNA hybridization efficiency [15, 16].
Covalent bonding	DNA is immobilized on the surface of electrodes through the formation of covalent bonds such as amide bonds, ester bonds, ether bonds, Au-S, and Ag-S [15, 16].	Good stability, flexible structure, high efficiency of DNA immobilization and hybridization, and high binding strength.
Avidin/streptavidin-biotin interaction	Another strategy for non-covalent immobilization of DNA probes on the electrode surface is based on the formation of complexes of avidin (either streptavidin)-biotin [15, 16].	The method is simple, stable and resistant to extreme temperatures, pH, denatured detergents, and organic solvents [15, 16].

DNA fragments must be immobilized in a manner that maintains stability, reactivity, accessibility to the target analyte, and optimal orientation. An electrical signal is generated when the target DNA binds to the complementary sequence of the capture or DNA probe in a process called hybridization [13]. The occurrence of this specific hybridization can be detected using an electrochemical transducer [6]. Electrochemical DNA biosensors can be label-based (indirect detection) and label-free based (direct detection).

Label-based electrochemical DNA biosensors (indirect detection)

For label-based electrochemical DNA biosensors (indirect detection), numerous studies introduced redox-active molecules as indicators to promote electron transfer between the electro-active base and the electrode surface. DNA was detected indirectly by measuring the electrical signals generated by the modified substances [7].

The incorporation of redox-active indicator onto the DNA surface is the most popular way to monitor the DNA hybridization events *via* an electrochemical method. In this detection strategy, the hybridized DNA-modified electrode is soaked in the buffer solution containing the redox-active indicator for a certain period to allow the interaction between DNA and redox indicator molecule [17]. Selection requirements for redox-active indicators are summarized below: (a) Indicators cannot affect the activity of bioreceptors and cannot react with the electrode material itself. (b) They should possess the ability to bind with ssDNA or dsDNA selectively [13]. Moreover, the redox-active indicator should possess a high reversibility of electron transfer at a low potential, compatible with voltammetry response, high selectivity and sensitivity.

Since then, numerous types of redox-active indicators have been utilized; organic dyes such as methylene blue (MB), as a redox hybridization indicator for electrochemical DNA detection has become a popular choice for the development of electrochemical DNA biosensors. The use of MB as the indicator has a great advantage including of high discrimination of binding affinities between ssDNA and dsDNA, low potential, and environment-friendliness. MB displayed high affinities to the free guanine bases on ssDNA and the MB could bind the hybridized DNA surface *via* electrostatic interaction with negative-charged phosphate group of DNA or intercalative interaction between G-C pairing bases. Another redox-active indicator method is the utilization of redox-labeled DNA probe where MB would undergo the changes in conformation that could affect the efficiency of electron transfer to and from electrode surface upon the DNA hybridization events [14, 17].

A schematic diagram of an electrochemical DNA biosensor for base-thiolated DNA probe-labeled with MB on a gold electrode surface through a monolayer self-assembly technique is shown in Figure 1.

Label-free electrochemical DNA biosensors (direct detection)

For label-free electrochemical DNA biosensors, DNA's own structure and composition are of paramount importance for electrochemical detection. Among the four DNA bases, guanine and adenine moieties are the most electroactive bases that are easily oxidized and adsorbed within a certain potential range, while thymine and cytosine require higher potential for oxidation, and hence electron transfer can be performed directly on several electrode surfaces [2, 17]. Even the sensing strategy based on direct redox reaction of nucleic bases has high sensitivity; the large background current interference limits its application. The oxidation reaction of ribose destroys the phosphate backbone of DNA, and it is rarely used for DNA-modified electrodes [13, 18]. The schematic diagram of the label-free electrochemical DNA biosensor is shown in Figure 2.

Application of electrochemical DNA biosensor for virus detection

Viruses are generally harmful because they can cause disease. However, with the advancement of science and technology, viruses are often used in the fields of pharmacy, biotechnology, and agriculture [12]. Viral biosensors offer exciting alternatives to diagnostic assays and have several advantages such as simple instrumentation, and sensitive, rapid, and portable platforms when compared to conventional laboratory-based methods [19]. Biosensors are analytical devices that couple biological recognition elements such as nucleic

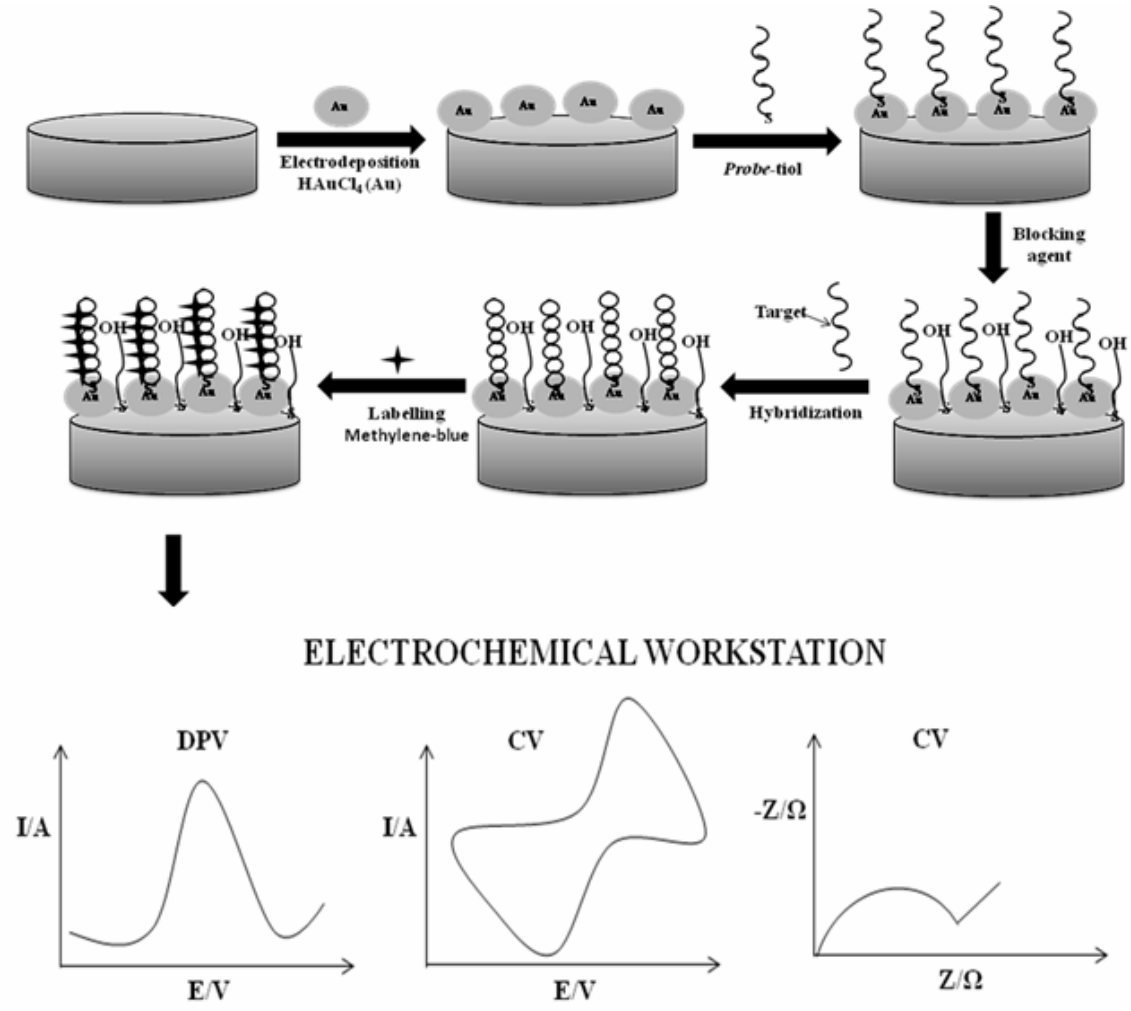

Figure 1. Electrochemical DNA biosensor for MB-labeled base thiolation DNA probe on gold electrode surface *via* monolayer self-assembly. The basic principle of electrochemical DNA biosensor is a DNA sequence (probe) immobilized on a transducer surface in order to recognize its complementary DNA target to form a DNA double helix formation. This hybridization event can be converted into a quantified signal by the transducer in the form of electrochemical for detection.

acids with a transducer that can detect the interaction of the analyte, and can be applied for medical diagnosis. Immobilization of DNA and working electrode material greatly affects the performance of the electrochemical sensor, because the target reacts on the working electrode. The resulting electrochemical signal is proportional to the concentration of the target and the electrochemical signal can be used with voltammetric, amperometric, and impedance biosensors [20]. A variety of electrochemical DNA biosensors have been developed for detecting different viruses (See Table 2).

Manzano *et al*. [36] reported the development of an electrochemical DNA biosensor for label-free detection of a specific nucleic acid sequence characteristic for hepatitis A virus (HAV). A disposable gold electrode was functionalized with the specific capture probe and tested on complementary ssDNA and on HAV cDNA. The DNA hybridization on the electrode was measured through the monitoring of the oxidative peak potential of the indicator tripropylamine by cyclic voltammetry. To prevent non-specific binding the gold surface was treated with 3% BSA before detection. Sensors designed to detect hepatitis A virus (HAV) showed a

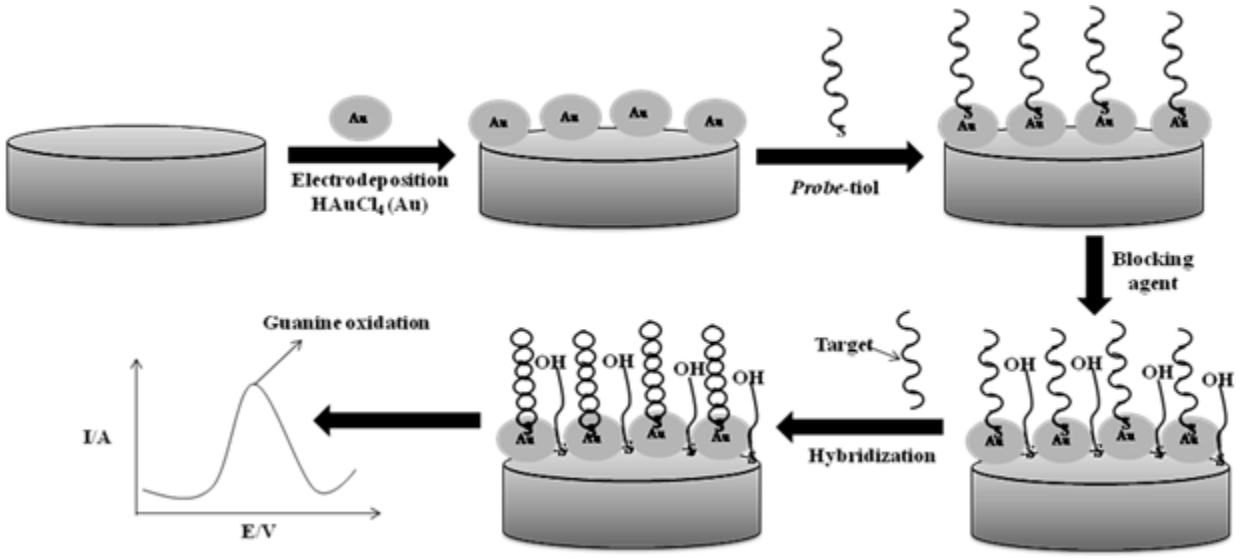

Figure 2. Label-free electrochemical DNA hybridization. The intrinsic electroactivity of guanine or adenine bases can be used for direct measurement of nucleic acids in the label-free assay.

limit of detection of 0.65 pM for the complementary ssDNA and 6.94 fg/μL for viral cDNA. For a comparison, nRT-PCR quantified the target HAV cDNA with a limit of detection of 6.4 fg/μL [52].

Ilkhani *et al*. [43] reported that frequent outbreaks of Ebola are worrying and continue escalating, mainly in Guinea, Liberia and Sierra Leone. Vaccine candidate tests are still being tested for the epidemic and could be available soon. Various diagnostic and therapeutic assays have been developed using recombinant viral vectors or antibodies that target the viral glycoprotein. However, innovative approaches are still needed, such as the development of electrochemical-based DNA sensing devices for DNA diagnostics of the Ebola virus. The electrochemical biosensor was developed by modifying the gold surface of the screen-printed electrode using single-strand DNA as a capture probe *via* Au-S bounds. A thiolated DNA capture probe sequence was immobilized on the screen-printed electrode surface and hybridized with biotinylated target strand DNA for the fabrication of Ebola DNA-sensing devices. Prior to the electrochemical detection of the enzymatic product by differential pulse voltammetry (DPV) method, the biotinylated hybrid was labelled with a streptavidin-alkaline phosphate conjugate on the surface of the working electrode. They optimized all the experiment steps using electrochemical impedance spectroscopy. From the experimental calibration curve, DPV current height versus complementary and non-complementary target strand DNA concentrations was plotted. The regression equation obtained from the calibration curve can determine the detection limit for complementary oligonucleotides of 4.7 nM. The obtained results indicated that the Ebola virus DNA can be detected using electrochemical biosensor with high reproducibility, sensitivity and selectivity. This method can be employed for the detection of Ebola virus DNA in real samples as a screening or initial method before further clinical analysis is carried out [43].

Chaibun *et al*. [48] reported the rapid detection of RCA (rolling circle amplification)-based SARS-CoV-2 S and N genes using DNA. This test involves the hybridization between of the RCA amplicons and probes that were functionalized using a redox active label which is then detected by differential pulse voltammetry (DPV). RNA and cDNA samples prepared from clinical samples were used as the template in RCA, using the one-step hybridization method and electrochemical detection. All 41 samples (11 RNA and 30 cDNA) prepared from SARS-CoV-2-positive clinical samples yielded positive results, while the 65 samples

Table 2. The development of the electrochemical DNA biosensor for virus detection.

Target	Electrode	Immobilization	Technique	LoD	Ref.
HIV	Gold Electrode	Thiol-based self-assembled monolayers (SAM)	Label-free	4×10^{-2} nM	[21]
	Reduce Graphene Oxide (rGO)-Glassy Carbon Electrode	Physical Adsorption	Label-free	3.0×10^{-13} M	[22]
	GR/AuNPCs-Glassy Carbon Electrode	Thiol-based self-assembled monolayers (SAM)	Label-methylene blue	30 aM	[23]
	Graphene-Nafion Composite Film	Physical Adsorption	Label-free	2.3×10^{-14} M	[24]
	Epoxy-Graphite Electrode	Surface Activation with EDC/NHS	Label-free	-	[25]
	Graphene Carbon Electrode	Thiol-based self-assembled monolayers (SAM)	Label-free	37 aM	[26]
	Glassy Carbon Electrode	Physical Adsorption	Label-free	1×10^{-16} M	[27]
	AuNP-Glass Fiber/ Carbonaceous Electrode	Surface Activation with EDC/NHS	Label-free	13 fM	[28]
	AuNP-Carbon Paste Electrode	Thiol-based self-assembled monolayers (SAM)	Label-free	0.3 fM	[29]
Virus Zika	AuNP-PET Electrode	Thiol-based self-assembled monolayers (SAM)	Label-free	25.0 ±1.7 nM	[30]
	Pencil Carbon Graphite Electrode	Surface Activation with EDC/NHS	Label-free	25.4 pM	[31]
	Reduce Graphene Oxide (rGO)-Graphite Electrode	Physical Adsorption	Label-free	0.1 fg/mL	[32]
	Glassy Carbon Electrode-AuNPs/SiPy	Thiol-based self-assembled monolayers (SAM)	Label-free	0.82 pmol/L	[33]
Hepatitis B	Carbon Paste Electrode-AuNP	Thiol-based self-assembled monolayers (SAM)	Label-free	3.1×10^{-3} M	[34]
	Carbon nanotube electrode	Physical Adsorption	Label-free	1 pM	[35]
	Screen Printed Carbon Electrode-AuNP	Thiol-based self-assembled monolayers (SAM)	Label-free	6.94 fg/µL	[36]
	ZnO-Nanowires Electrode	Thiol-based self-assembled monolayers (SAM)	Label-free	0.1 pM	[37]
	$Co_3O_4^-$ Glassy carbon	Physical Adsorption	Label-free	0.38 pM	[38]
	WO_3/In_2O_3 Nanowires	Covalent bonds	Label-free	1 fM	[39]

Table 2 continued..

H1N1	Glass electrode	Physical Adsorption	Label-Pottasium ferrocyanide	3.70 PFU/mL	[40]
	Screen Printed Electrode-AuNP	Surface Activation with EDC/NHS	Label-free	0.667 ng/mL	[41]
	Screen Printed Carbon Electrode	Physical Adsorption	Label-HRP	1.12 unit/mL	[42]
Virus Ebola	Screen Printed Electrode-AuNP	Streptavidin-biotin interactions	Label (biotinylated hybrid with a streptavidin-alkaline phosphate conjugat)	4.7 nM	[43]
Norovirus	Gold/iron-oxide nanoparticle-CNT	Physical Adsorption	Label-free	8.8 pM	[44]
Virus Papilloma	Gold Electrode	Thiol-based self-assembled monolayers (SAM)	Label-free	1.7 copies/mL	[45]
	Gold nanotubes	Physical Adsorption	Label-free	1 fM	[46]
	MWCNT- Reduce Graphene Oxide	Surface Activation with EDC/NHS	Label-free	1.3 nmol/L	[47]
Sars-CoV-2 Gen N & S	Screen Printed Carbon Electrode	Thiol-based self-assembled monolayers (SAM)	Label-methylene blue dan acridine oranye	1 copies/µL	[48]
Sars-CoV-2 Gen N	Printed Circuit Board	Physical Adsorption	Label-methylene blue	-	[49]
Gen RdRp	Interdigitated electrode	Covalent bonds	Label-free	10 nM	[50]
ORF1ab	Flex Printed Circuit Board-Graphene	Surface Activation with EDC/NHS	Label-free	100 fg/mL	[51]

(40 RNA, 25 cDNA) prepared from SARS-CoV-2-negative clinical samples recorded negative results [44].

Target amplification by RCA followed by electrochemical biosensor detection requires three steps of target recognition and hybridization to its complementary sequence which is produced because the amplification product has multiple binding sites. First, the gene target must be hybridized along with the complementary sequence in the circular DNA, followed by ligation of the circular DNA to produce a Padlock DNA. After amplification of the Padlock DNA by phi29 DNA polymerase, RCA amplicons are produced, which are long repeats of the complementary sequence of the Padlock DNA. Next, the RCA amplicons are bound by the reporter and probes captured, followed by electrochemical detection of the redox-active dye. This strategy ensures high specificity is achieved using the assay. Moreover, utilization of magnetic capture and separation of targets from non-targets reduce the chances of carry-over contamination and pipetting error [44].

The complementary DNA (cDNA) or RNA target will be specifically hybridized using the biotinylated probe on the magnetic beads *via* hydrogen bonding, permitting their precise targeting. Moreover, this approach has become a valuable method because

Table 3. The development of CRISPR DNA biosensor technology for electrochemical virus detection.

Target	Type of CRISPR	Electrode	Technique	LoD	Ref.
Human Papillomavirus 16 (HPV16)	CRISPR-Cas12a	GE	Label-methylene blue	6,0 x 10^2 fg/mL	[54]
	CRISPR-Cas12a	GE	Label-methylene blue	2,8 x 10^6 fg/mL	[56]
HIV	CRISPR-Cas12a	GE	Label-spherical nucleic acid	30 fM	[57]

of their low cost, compatibility with liquid sample, and high surface area for hybridization [50]. The two genes were detected with different redox dyes (methylene blue for N gene and acridine orange for S gene); the strength of the current signals obtained from both genes were very similar. The similarity of the signals was attributed to the same mechanism of the redox reaction (two-electron oxidation) for both dyes. The detection limit of both the N and S genes was 1 copy/μL, with a linear range of 1 to 1×10^9 observations/μL. Therefore the detection limit using this method is smaller than that obtained with the CDC RT-PCR test of 3.2 copies/μL [44].

Bonini *et al*. [53] reported that a method that can be used to modify genes (DNA) in living organisms is being developed, called CRISPR. The DNA flanked by two or more CRISPRs is part of the viral DNA called foreign DNA. Then there is a group of proteins or enzymes called Cas or CRISPR-associated proteins, which cleave DNA and remove invading viruses. CRISPR/Cas systems have been rapidly adapted as a recognition element in the development of biosensors and biosensing systems for the detection of nucleic acids, which are important targets in molecular diagnosis [53].

In 2019, Dai and co-workers first used Cas12a's assurance activity in a labeled electrochemical biosensing system called electrochemical CRISPR (E-CRISPR). Each CRISPR array is associated with a DNA module (adapter and effector) that encodes various Cas proteins such as helicases and endonucleases. Biosensing systems exploit the Cas effector to recognize a specific target in solution and the collateral activity to cleave nucleic acids on the electrode surface. They immobilize non-specific short ssDNA characterized by redox mediator methylene blue (MB-ssDNA) on the surface of the gold electrode, and then use Cas12a/crRNA complex to recognize dsDNA targets in solution and trigger collateral activity, leading to cleavage MB-ssDNA on the electrode surface. Electrochemical signal disconnection is measured by voltammetry thanks to the residual MB redox mediator on the electrode surface [54].

Heo *et al*. [55] reported the development of an electrochemical biosensor based on the trans-cleavage activity of CRISPR/Cas13a for rapid, sensitive, and nucleic-acid-amplification-free detection of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). A redox probe conjugated with ssRNA is immobilized on the electrode surface modified with a nanocomposite and a nanoflower. The SARS-CoV-2 RNA is captured by the Cas13a-crRNA complex, which triggers the RNase function of Cas13a. The enzymatically activated Cas13a–crRNA complex is subsequently introduced to the reRNA-conjugated electrochemical sensor, and consequently cleaves the reRNA. A change in current occurs due to the release of the redox molecule labeled on the reRNA, which is trans-cleaved from the Cas13a–crRNA complex. The biosensor can detect as low as 4.4×10^{-2} fg/mL and 8.1×10^{-2} fg/mL of ORF and S genes, respectively, with a linear range of 1.0×10^{-1} to 1.0×10^5 fg/mL.

A major advantage of CRISPR diagnostics is that they can be highly specific. There are several developments of electrochemical DNA biosensors reported using the CRISPR/Cas system which can be seen in Table 3.

CONCLUSIONS

Based on the above review, the rapid development in the field of biosensors over the past decades provides a new perspective to investigate the application of biosensors for virus detection. Due

to the advantages of being portable and being able to provide faster results compared to conventional methods, the biosensor method is used for virus detection using DNA. Biosensors combine a bioreceptor with a transducer. Single-stranded DNA (ssDNA) probes immobilized on a electrode surface in order to recognize its complementary DNA target to form a DNA double helix formation. The probe is associated with a transducer translating a hybridization event into a physically measurable value. Electrochemical detection of DNA hybridization is usually involves monitoring the current at a constant potential. DNA hybridization detection methods can be classified into label-based and label-free methods. For label-based, methylene blue (MB), as a redox hybridization indicator has become a popular choice for the development of electrochemical DNA biosensors. The use of MB as the indicator has a few great advantages including high discrimination of binding affinities between probe and target, low potential, and environment-friendliness. For label-free, guanine is a nitrogenous base that is more easily oxidized because it has a lower standard reduction potential value than other nitrogenous bases, hence electron transfer can be performed directly on several electrode surfaces. Redox reaction of nucleic bases has high sensitivity and small background current disturbances because there is no interference due to indicators; therefore label-free DNA hybridization is a direct detection method that is more widely developed for DNA electrochemical biosensors for virus detection.

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CONFLICT OF INTEREST STATEMENT

Nothing to declare.

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