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Short Review

HIV Biosensors – The Potential of the Electrochemical Way

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Because of high costs and time consuming common HIV tests, new ways in diagnosis, offering rapid, cheap, and accuracy possibility are looking for. One of the most promising fields is electrochemistry, providing very good specifity, relatively low price, sensitivity, and possibility of miniaturization. Good sensitivity of electrochemical methods also facilitates the detection of HIV virions during the diagnostic window, i.e. the period when HIV antibodies are produced, but under detection limits of common diagnostic methods. In this review, the advantages of electrochemical HIV biosensor are summarized. Moreover, the potential of electrochemistry compared with other conventional methods is discussed.

Keywords: Human Immunodeficiency Virus; Biosensor; Lab-on-a-Chip; Electrochemical detection; Electrode modification

1. HUMAN IMMUNODEFICIENCY VIRUS

Human immunodeficiency virus (HIV) is a lentivirus that causes the immunodeficiency syndrome (AIDS). HIV disease was initially reported in 1981 followed by the identification of the HIV as the cause of the disease in 1983. HIV is a global pandemic that has become the leading infectious disease killer of adults worldwide [1-3]. The AIDS pandemic continues to spread unchecked in many parts of the world with greater than 34 million individuals currently infected with HIV. To date, HIV is divided into HIV-1 and HIV-2 strains [4,5], whereas HIV-2 represents a significant minority of all HIV infections in some countries [6]. While similar in many ways, there are important differences between HIV-1 and HIV-2 that provide insights into virus evolution, tropism and pathogenesis. Major

differences include reduced pathogenicity of HIV-2 compared to HIV-1, enhanced immune control of HIV-2 infection and often some degree of CD4-independence. One of the major characteristics of HIV-1 is its high genetic variability and extensive heterogeneity. This characteristic is due to its molecular traits, which in turn allows it to vary, recombine, and diversify at a high frequency. As such, it generates complex molecular forms, termed recombinants, which evade the human immune system and thus they survive [7]. Diagnostic tests have focused on HIV-1-associated biomarkers, including host cell integrated proviral DNA [8-10] and the viral capsid protein antigen p24 presence [11]. Of the currently available diagnostic technologies, PCR-based methods predominant, or various methods for determination of p24, such as radioimmunoassay [12], fluoroimmunoassay [8], enzyme-linked immunosorbent assay [13], and electrochemical methods [9]. In this review, we attempted to summarize the advantages and disadvantages of the last mentioned detection technique called electrochemistry.

2. ELECTROCHEMISTRY BIOSENSOR FOR DETECTION HIV

Good sensitivity of electrochemical methods facilitates detection of HIV virions during the diagnostic window, which is the period when HIV antibodies are produced. Therefore, we mainly discuss the novel electrochemical methods for detection of HIV published recently.

2.1. Electrochemical biosensors for detection of HIV sequence

In recent years, various studies have been performed to identify the HIV. An electrochemical DNA biosensor was developed with covalent immobilization of HIV probe for single-strand DNA (ssDNA) on the modified glassy carbon electrode (GCE). This method is based on the studying of the electrochemical behavior of aquabis(1,10-phenanthroline)copper(II) perchlorate [Cu(H_2O)(phen)(2)]center dot 2ClO_4, where phen =1,10-phenanthroline, after its binding to DNA at the GCE and in solution. [14-16]. The employment of these electrochemical hybridization indicators could provide a simple, and rapid detection and might have a promising future in transducing DNA hybridization and for diagnosis HIV and AIDS. Kerman et al. described an electrochemical biosensor with a new bioorganometallic approach for detection of HIV-1 protease (HIV-1 PR) using surface-bound ferrocenoyl (Fc)-pepstatin conjugates [17]. In another study, nano-MnO_2/chitosan composite film modified glassy carbon electrode (MnO_2/CHIT/GCE) was fabricated and DNA probe was immobilized on the electrode surface. The immobilization and hybridization events of DNA were characterized by cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The EIS was applied to the label-free detection of the target DNA. The HIV gene fragment was successfully detected by this DNA electrochemical sensor [18]. Zhang et al. designed and fabricated a simple, inexpensive and stable multi-electrode array and developed a label-free electrochemical DNA biosensor array for the simultaneous detection of HIV-1 and HIV-2 as a model system of multiplexed DNA sequences-specific analysis using microliters of sample. This novel multi-electrode array was

comprised of six gold working electrodes and a gold auxiliary electrode, which were fabricated by gold sputtering technology, and a printed Ag/AgCl reference electrode fabricated by screen-printing technology [19]. A simple and ultrasensitive electrochemical DNA biosensor using long-range self-assembled DNA nanostructures as carriers for signal amplification with two auxiliary probes was designed by Chen et al. A cascade of hybridization events between the two auxiliary probes can lead to long-range self-assembly and form micrometer-long one-dimensional DNA nanostructures. In the presence of target DNA, each copy of the target can act as a trigger to connect a DNA nanostructure to a capture probe on the electrode surface. Then, a great amount of redox indicator $[Ru(NH_3)_6]^{3+}$ can be electrostatically bound to the DNA nanostructures and eventually result in significantly amplified electrochemical signals [20].

An innovative polymer lab-on-a-chip (LOC) for reverse transcription (RT)-polymerase chain reaction (PCR) was designed, fabricated, and characterized for point-of-care testing (POCT) clinical diagnostics. In addition, a portable analyzer that consists of a non-contact infrared (IR) based temperature control system for RT-PCR process and an optical detection system for on-chip detection, was developed and used to monitor the RT-PCR LOC. The newly developed LOC and analyzer were interfaced and optimized for performing RT-PCR procedures and chemiluminescence assays in sequence. As a clinical diagnostic application, HIV was successfully detected and analyzed using the newly developed LOC and analyzer, where the primer sets for p24 and gp120 were used as the makers for HIV [21]. Another team designed the lab-on-a-chip for the assaying of HIV, which includes the sample preparation, reaction, and signal amplification module. A laser induced fluorescence system was suggested for real-time monitoring of the signals [22].

Nowadays, diamond is a promising material for merging solid-state electronics and bioelectronics that can be coupled with DNA molecules, proteins and cells. In recent years, many applications of biosensing using diamond as a material and electronically active devices have been developed. Ruslinda et al. showed an aminated diamond-based RNA aptasensor for HIV trans-activator of transcription (Tat) peptide-protein detection. The immobilized procedure was based on the binding interaction between positively charged amine terminated diamond and the RNA aptamer probe molecules with the negatively charged surface carboxylic compound linker molecule such as terephthalic acid [23]. Ruslinda et al. also reported the contribution of HIV-1 Tat protein to the electronic response of a diamond FET-based RNA aptamer to the electronic properties of diamond with the aim of clarifying the electronic response of such biosensors. The reliable use of a real sample of HIV-1 Tat protein by a diamond-FET-based RNA aptamer was demonstrated for the first time, and showed the potential of diamond biointerfaces for adaption to the clinical monitoring and diagnostics [24].

2.2. Electrochemical biosensors for detection of HIV peptides and/or proteins

The HIV-1 capsid protein, the p24 antigen (p24), has great significance for diagnostics, because it can be detected several days earlier than host-generated HIV antibodies, which are the target of almost all current tests used in the field, and can be used to design sensitive assays without the need

of PCR [25]. Gan et al. showed an ultrasensitive electrochemical immunosensor for HIV p24 based on Fe_3O_4@SiO_2 nanomagnetic probes and nanogold colloid-labeled enzyme–antibody colopolymer as a signal tag [26], and another type based on mercapto succinic acid hydrazide copper monolayer modified gold electrode [27]. Other authors also showd that amperometric immunosensor based on a polyelectrolyte/gold nanoparticle magnetic method is an effective and high performance for detection of HIV p24 in serum [28-32]. We show the general scheme of these methods in **Fig. 1**. Picomolar electrochemical detection of HIV type-1 protease (HIV-1 PR) using ferrocene (Fc)-pepstatin-modified surfaces was described by Mahmoud et al. Gold electrode surface was modified with gold nanoparticles (AuNP) or thiolated single walled carbon nanotubes/gold nanoparticles (SWCNT/AuNP). This sensing electrode modification showed remarkable detection sensitivity to the presence of the protein [33]. An ion channel biosensor was described for label-free detection of inhibitors, which bind to the coiled coil domain of HIV type 1 gp41, where gp41 is the viral transmembrane glycoprotein responsible for fusion between HIV-1 and host cells. Nanomolar quantities of peptides and small molecules, which bind in the hydrophobic pocket, could be selectively detected, providing a method for label-free detection of binding to gp41 [34].

Labid et al. described the preparation of a thin film of a ferrocene-labeled lipoic acid (Fc-LA) derivative on a screen-printed carbon electrode functionalized with gold nanoparticles (GNPs-SPCE), which is then modified with short peptide VEAIIRILQQLLFIH to bind to the reverse transcriptase (RT) of HIV-I. This modified electrode is then used to detect HIV-1 RT in human serum by square-wave voltammetry [35,36]. Recently, Shafiee et al. demonstrated for the first time a label-free electrical sensing method that can detect lysed viruses, i.e. viral nano-lysate, through impedance analysis, offering an alternative technology to the antibody-based methods such as dipsticks and Enzyme-linked Immunosorbent Assay (ELISA). The presented method is a broadly applicable platform technology that can potentially be adapted to detect multiple pathogens utilizing impedance spectroscopy for other infectious diseases including HIV and also herpes, influenza, hepatitis, pox, malaria, and tuberculosis [37].

Biosensors are good not only for the identification of the virus, but also have great value for studies of drug and advances in medicine and pharmacology to treat HIV. Kang et al used HIV Reverse Transcriptase (RT)-immobilized AlGaN/GaN high electron mobility transistors (HEMTs) and binding-site models to find out the dissociation constants of the HIV RT-inhibitor complex and the number of the binding sites on RT for the inhibitor called Efavirenz. The AlGaN/GaN HEMTs and the binding-site-models are demonstrated to be good tools to assist drug developments by the elucidating the dissociation constants and the number of binding sites, which can largely reduce the cost and time for drug developments [38].

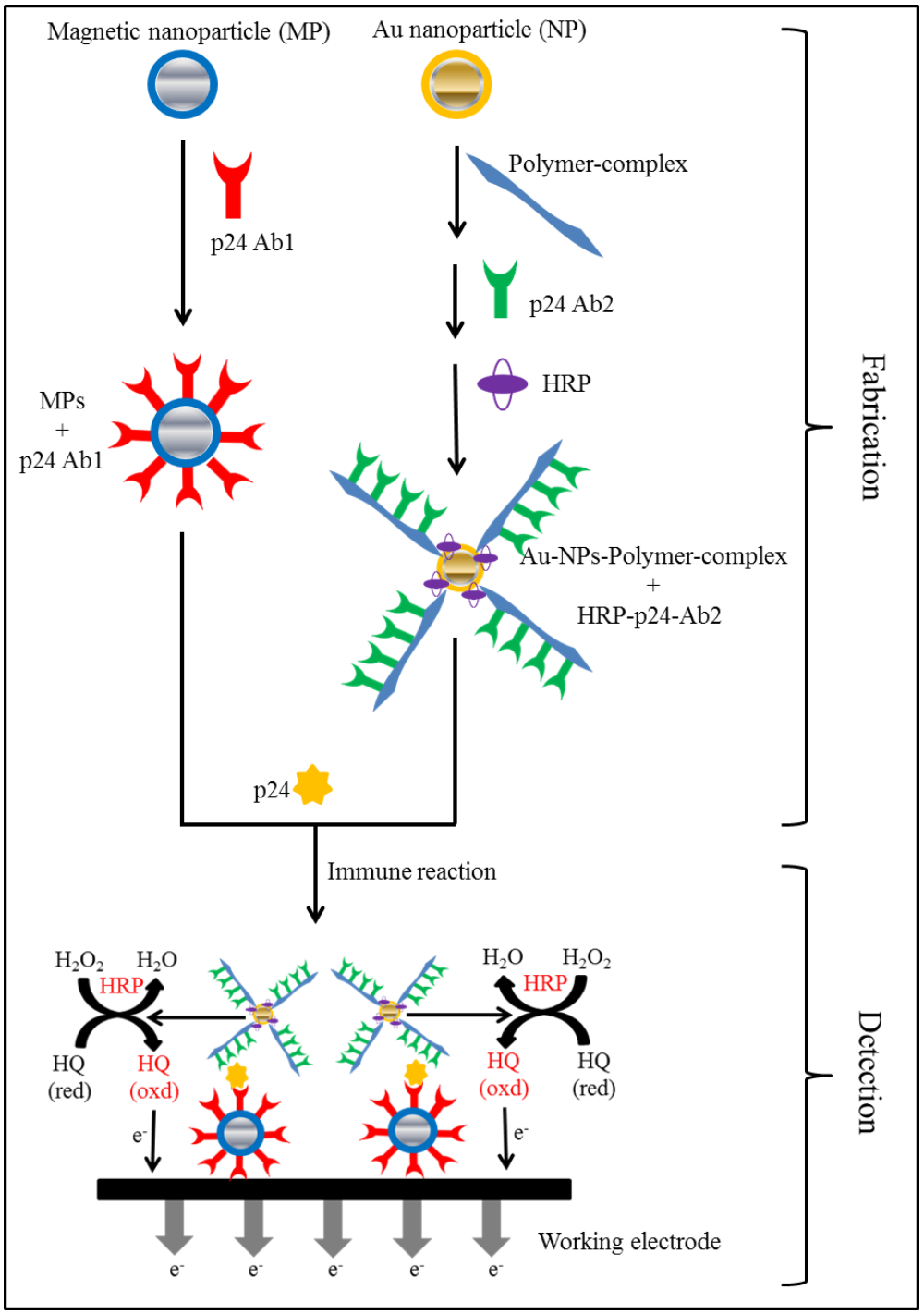

Figure 1. General scheme of the fabrication and detection procedure of electrochemical immune-biosensor HIV antigen p24. Enzyme–polymer complex with Au nanoparticles (Au-NPs) contains molecules of horseradish peroxidase (HRP) and molecules of the secondary antibody of p24 (p24 Ab2). The Magnetic nanoparticles (MNPs) contains molecules primary antibody of p24 (p24 Ab1). A large amount of HRP, which favors the oxidation of HQ by H_2O_2, provides a greatly amplified signal.

3. WHY USE ELECTROCHEMICAL METHODS THAN OTHER CONVENTIONAL METHODS FOR DETECTION AND/OR IDENTIFICATION HIV

The biosensors have been widely applied in various fields for analyte detection due to their simplicity, high sensitivity, and potential ability for real-time and on-site analysis. The typical biosensor is tailored for detection of specific viruses, often using optical or electrochemical transduction systems. In this review, we attempt to show that electrochemistry biosensor methods have great advantages than other conventional methods for detection and/or identification HIV.

3.1. DNA biosenzors

The electrochemical DNA biosensors, which were developed with covalent immobilization of HIV probe, had numerous factors affecting the probe immobilization, target hybridization, and indicator of binding reactions to maximize the sensitivity and speed of the assay. Once you had known the optimal conditions, the utility of the new electrochemical hybridization indicator could provide a simple, rapid detection and might have a promising future in transducing DNA hybridization. The electrochemical DNA sensor developed might have the potential application to diagnosis of diseases. Among them, the use $[Co(phen)2IP] \cdot 2ClO_4 \cdot 3H_2O$ as a new electrochemical hybridization indicator, selective detection of the complementary ssDNA using electrochemical DNA sensor could be achieved. The surface hybridization of the immobilized single-stranded HIV DNA fragment with its complementary DNA fragment was evidenced by electrochemical method using $[Co(phen)_2IP]_2^+$ as a novel electrochemical indicator with a detection limit of 27 pmol [14-16]. In addition, Au electrode was showed to provide a highly sensitive virus sensor to detect HIV-1 in a label free system due to the excellent electrical and mechanical properties of fabricated Au nanoparticle. HIV-1 were successfully quantified from 600 $pg \cdot mL^{-1}$ to 375 $pg \cdot mL^{-1}$ [30]. Therefore, one of the main advantages of electrochemical DNA biosensors is the detection limit and short time for the identification and/or detection of HIV. Another reason for use of electrochemical methods is the fact that other conventional methods for detection and/or identification sequences of HIV is minimized cross-contamination [21]. The method used the immobilized procedure based on the binding interaction between positively charged amine terminated diamond and the RNA aptamer probe molecules serves not only as labeled or label-free detection but also provides a platform for a simple, sensitive, and selective detection of proteins [23,24].

3.2. Immuno biosensors

The electrochemical immunosensor is one of the most attractive analytical tools due to several advantages such as specificity, simplicity, direct detection and time savings of the analyses compared with conventional immunoassay techniques [39-41]. Electrochemical biosensors for detection peptide and/or protein of HIV showed more advantages than other conventional methods for detection and/or identification of the virus. Typical detection methods for the diagnosis of blood-borne pathogens, such as HIV, as established widely in medical laboratories, include ELISA. However, ELISA requires

specific reagents, such as buffers and enzymes, and equipment, and due to this it is hardly used *in situ*. The electrochemical immunosensor for HIV p24 antigen detection showed an ultrasensitive biosensor, whereby the detection sensitivity was 1000 times higher compared to ELISA based assay [26]. Some procedures for detection of HIV relying on immunoassay recognition of HIV antibody (anti HIV) are available, but in the early stages of HIV infection when anti HIV has not yet appeared in the sufferers serum (the window period), the patient is highly infectious. If detection during this period is attempted, there is a great possibility to miss patients suffering from HIV. Nevertheless, one of the diagnostic markers of HIV glycoprotein antigen (p24) was found in human serum in the "window period", although its content is very low (<5 ng/mL) in the early one week after infection. If p24 is found at this time, HIV can be diagnosed, which will effectively shorten the window period [29]. Conventional immunoassay methods need enzyme or fluorescent-labelled antibody/antigen, not to mention its lengthy analysis that requires highly skilled personnel, specially equipped laboratories, and expensive chemicals. Therefore, the electrochemical immunosensor is new and novel method for fast and convenient monitoring of p24 [42,43].

4. FUTURE PERSPECTIVES OF ELECTROCHEMICAL BIOSENSORS

The electrochemical biosensors have witnessed an escalating interest nowadays, both in the research and commercial fields. These instruments can be applied including those aimed at the presence of nucleic acid or peptide from HIV. In summary, we present in this review that development of electrochemical biosensors for detection HIV is novel field of interest with great advantages to be used for used in resource-limited settings, as the development of rapid, accurate, and portable diagnostic tools. These types of tools might be beneficial for many purposes such as fast on site analysis of many potential blood donators especially when natural disaster or similar medical attention attracting event occurs. Recent efforts in diagnostics development for the identification and/or detection of HIV are beginning to produce solutions that could be used in the low-resource setting for developing countries. In particular, these electrochemical methods have the potential to be affordable for even the lowest-resource settings. Therefore, electrochemical sensors methods can be considered as a new tool for identification and/or detection of HIV, representing a wide field of possible applications, like clinical diagnostics and treatments research of HIV.

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CONFLICT OF INTEREST:
The authors have declared no conflict of interest.

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