



Review Article

Advancements in Biosensing Technologies for the Detection of Human Immunodeficiency Virus

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Abstract

HIV (Human Immunodeficiency Virus) is one of the major health concerns and continues to be an epidemic causing threat to a large population of the world. Despite great efforts and strategies being employed for containing its spread, it still is hard to tackle in resource-limited settings. Conventional molecular detection methods are laborious and require trained staff and well-equipped laboratory facilities. In this review we have discussed biosensors for rapid, portable and highly sensitive point-of-care detection providing timely diagnosis and monitoring of infectious agents like HIV. Different types of biosensors for the detection of HIV have been developed over the last twenty years including Electrochemical, Optical and Piezoelectric biosensors. Although biosensors provide various advantages over standard diagnostic methods still there are technical limitations that need to be addressed for their commercial implementation.

Keywords: Biosensor; Virus; Human Immunodeficiency virus; Detection; Diagnosis

Introduction

HIV (Human Immunodeficiency Virus) emerged under the evidence from Bushmeat theory, stating HIV similarity to SIV (Simian Immunodeficiency Virus) in chimpanzee and its transfer from apes/monkeys to humans [1]. With the start of colonization in Africa, HIV egressed epidemically as result of antibiotic era, use of unsterile injections, genital ulcer diseases and evolving sexual activity. HIV (human immunodeficiency virus) is a virus that attacks cells involve in fight against infection. HIV uses the elaborative cytokines system to replicate and inserts itself into human immune system by burdening the CD4+ T cells with virus and progression of disease. Body fluids that include blood, semen, vaginal and rectal fluids, and breast milk are means of HIV transmission. Symptoms of HIV infection vary depending on stage of infection with swollen lymph nodes, regular fever or sudden weight loss at later stages [2].

HIV is classified in the genus Lentivirus and belongs to the subfamily Orthoretrovirinae within the Retroviridae family

[3]. HIV is further characterized into type 1 and type 2 based on genetics and differences of the viral antigens. The surface glycoprotein (gp120) of HIV attaches to the CD4 receptor and a co-receptor (CKR5) on the surface of the host cell [4]. HIV has infected 75 million people and caused 32 million deaths since its start. At end of 2018, approximately 37.9 million people are living with HIV. Despite prevention measurements, HIV cost lives of 770,000 people and 1.7 million people were newly infected globally [5]. Figure 1.



Figure 1: Graphical abstract.

Different detection strategies are used for assessment of patients and infection status. The laboratory based detection methods involve Antibody screening, ELISA (enzyme linked immunosorbent assay), Western blotting that aims for antibodies to specific HIV proteins, p24 antigen detection using monoclonal or polyclonal antibodies and PCR based assays [6,7]. These techniques require trained technicians, multiple steps and powered laboratory equipment. Also, these methods involve the use of injection in earlier stages which brings concerns to sterility issues. Existence of trypanophobia more commonly among children and older people has limited wide use of these techniques. Early diagnosis of HIV is necessary for survival and proper treatment of infected people. Repeated testing and follow up consultation due to unclear results is extremely stressful for patient. Therefore, a portable and rapid device for an early diagnosis of virus is need of the hour.

Biosensors are defined as devices that use a biological recognition element integrated with a physicochemical transducer to provide specific qualitative or semi-quantitative analysis [8]. Leland C. Clark is called the “father of biosensors” because of his invention of a device that could measure oxygen in blood, water and other liquids [9]. Biomedical sensors can be used for the detection and monitoring of many medical conditions. Recently there has been an increase in demand for disposable and simple devices that are cost effective, user-friendly and give fast response time [10]. The applications of biosensors in disease diagnosis are vast, ranging from diabetes, cardiovascular diseases to virus detection. The emphasis of this review is on the different biosensor technologies for the detection of HIV. An overview of the biosensing system is given and different biosensor-based detection strategies for HIV are discussed.

Biosensors

Biosensors are integrated devices which are capable of quantitatively or qualitatively recognizing different molecules with the use of biological sensing components connected to a physicochemical transducer to produce a signal which is further detected by the help of a detector [11].

There are numerous applications of biosensors including detection of various diseases, monitoring of pollutants, detection of biomolecules, drug discovery, food safety and many more [12]. The IUPAC defines that a biosensor is a self-sufficient integrated device which has the capability to provide specific quantitative or semi-quantitative data using a bioreceptor that is in direct contact with a transducer. A biosensor is clearly differentiated from a bioanalytical system, requiring extra processing steps, like reagent addition [13].

General design of a biosensor

Biosensors generally are composed of the following basic components: a biorecognition element or bioreceptor, a transducer, signal processing element and a display or detector [14].

Bioreceptor

A bioreceptor is the component that specifically recognizes and binds to the analyte of interest and is very sensitive. It is mostly an immobilized biological element such as an enzyme, nucleic acids, antibodies or living cells [15].

Analyte: Analyte is the element of interest that is being monitored or measured in a biosensor.

Transducer: A transducer is the element that converts the reaction of biosensing into a measurable signal for instance an electrical or optical signal. The amount of signal produced corresponds to the analyte-bioreceptor interaction that is taking place [16].

Electronic System

The Electronic system of a biosensor consists of two parts: the first part is the signal processing unit of the biosensor that amplifies and processes the signal from the transducer whereas the second part is the Display unit that provides the data in a readable and user-friendly form [17] Figure 2.

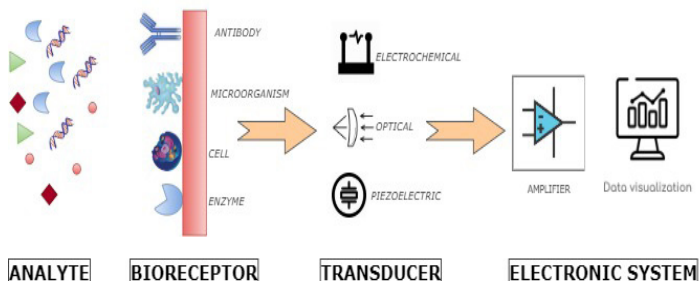


Figure 2: General Design of a Biosensor [18].

Types of biosensors

Biosensors are classified in two different ways, one is according to the mode of signal transduction and other is the different biorecognition elements. On the basis of transducers, biosensors can be categorized as electrochemical, electrical, piezoelectric, optical and thermometric sensors¹⁹. Each type has further subtypes as well. While on the basis of bio-recognition element, they can be divided into enzymatic biosensors, immuno-biosensors, whole-cell biosensors and DNA biosensors [20], Figure 3.

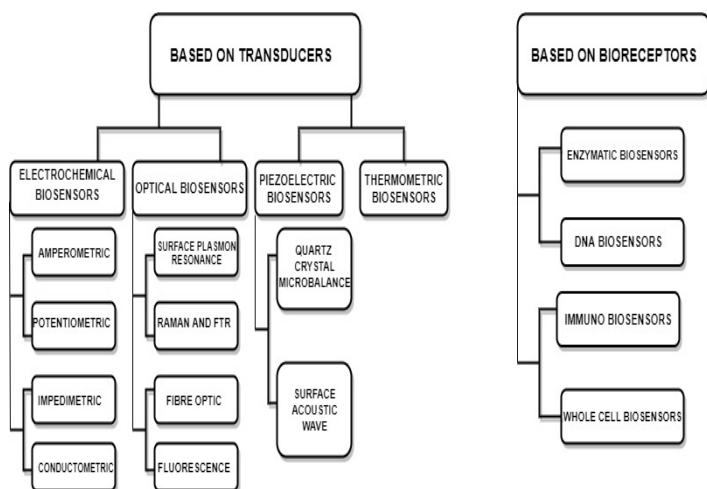


Figure 3: Classification of Biosensors [21].

Different biosensing platforms for the detection of hiv

Biosensors are small, portable devices that are specific, rapid and user-friendly. These qualities make them desirable for disease detection and monitoring. Biomolecules or biomarkers that are indicators of specific diseases such as cancer can be detected by using biosensors [22]. Sensitive and specific devices that can give early diagnosis are essential for increasing survival rates of patients. In the last decades the demand for simple detection methods with readily available results has increased a lot. Biosensors have been developed for many diseases like cardiovascular diseases, cancer, diabetes, hepatitis and other diseases caused by different viruses [10]. Similarly there are also many applications of biosensors for the detection and monitoring of HIV, some of which are mentioned in this section of the article.

Electrochemical biosensors for HIV

Electrochemical biosensors involve the monitoring of electrochemical reaction by checking the changes in current, impedance, voltage or resistance through electrode interface [23]. Electrochemical reaction could be an enzymatic reaction or capture of specific biological targets [24].

In these type of biosensors the reaction being studied can generate measurable potential, measurable current, charge accumulation or can detectably change the conductive properties amid the electrodes [25].

Zheng, et al. developed a sandwich amperometric immunosensor for the detection of HIV protein in which the anti-p24 antibodies were used to modify the electrode. Then horseradish peroxidase was used for labeling as a secondary antibody. Hydrogen peroxide and hydroquinone solution was used for immersing the electrode for signal production. The detection limit of this assay was 0.008 ng/mL which is much better when

compared to conventional methods [26].

A molecular imprinted polymer based electrochemiluminescence biosensor was developed by Babamiri et al. for the detection of HIV-1 gene. In this assay o-phenylenediamine was used as a functional monomer and HIV aptamer as a template. A linear detection of HIV gene (0.3fM to 0.3 nM) was observed. Compared to non-complimentary sequences this biosensor showed better specificity [27].

Shafiee, et al. fabricated an impedance biosensor to check impedance changes for studying the HIV viral nano-lysates. Magnetic beads that were coated with anti-gp120 antibodies were used to capture HIV-1 subtypes. This step was followed by viral lysis. The viral nano-lysate samples were used then for analysis of impedance by a microdevice with two gold electrodes. Changes in impedance of multiple HIV-1 subtypes and control were evaluated. This device showed that HIV-1 samples produced different impedance values when compared with controls [28].

An electrochemical biosensor with graphene electrode modified with gold nanocluster (GR/AuNCs) was developed by Wang, et al. for HIV DNA detection using exonuclease III-assisted target recycling amplification approach. Aptamers with cytosine-rich base were used as capture probes for constructing the biosensor. Through this platform, high sensitivity and good selectivity detection of target HIV DNA was achieved with a detection limit of 30 aM. Also when tested at 10 fM, the HIV target probe revealed 99.8 % of a recovery rate during human serum sample analysis [29].

An ultrasensitive electrochemical biosensor was fabricated by Li et al. for the detection of HIV gene in human serum samples based on Differential Pulse Voltammetry (DPV) analysis. The DPV platform consisting of Glassy Carbon Electrode (GCE) the surface of which is modified with amino-reduced graphene oxide (NH₂-rGO) and β -cyclodextrin (β -CD) to aid in the complementary HIV gene sequence (capture DNA) binding. Methylene blue dye which binds to single stranded DNA generated a stronger DPV signal in the absence of the HIV gene whereas when HIV gene was present it was hybridized to capture DNA sequence to form a double stranded DNA eventually generating a weaker signal. The detection limit of this biosensor was lower (8.7fM) with high electivity [30]. Similarly another HIV biosensor using Glassy Carbon Electrode was fabricated by researchers at China, modifying it with polyaniline/graphene nanocomposite material. Single stranded DNA was then immobilized on the surface of electrode which can hybridize to the target HIV-1 gene. As a result of this hybridization, a change in impedance value was observed by electrochemical impedance spectrometry (EIS) which corresponds to the concentration of HIV-1 gene. This device depicted a low detection limit of about 1.0×10^{-16} M [31], Figure 4.

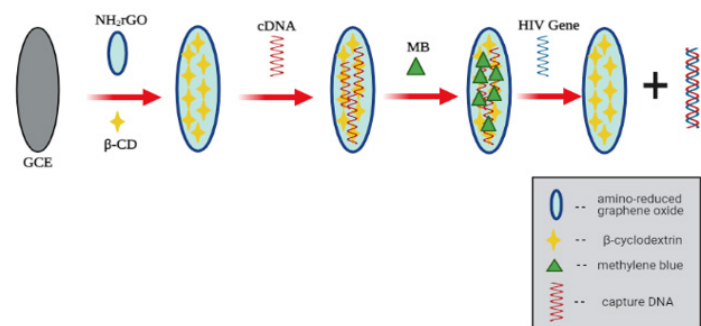


Figure 4: Schematic diagram of the DPV based electrochemical biosensor [30].

Optical biosensors for HIV

Label-free optical photonic crystal biosensors are sensitive, speedy and gives reliable detection of a number of targets such as proteins, cells and viruses by observing the changes in dielectric permittivity [32]. Shafiee, et al. demonstrated an optical sensing photonic crystal biosensor for detection of HIV-1 from biological samples. It was studied that, there was a shift in the resonant peak wavelength value when the virus was adsorbed by the surface. This biosensor can even detect low concentrations of the virus. They also observed HIV-1 in plasma samples and phosphate buffered saline samples with viral loads in range of 10^4 to 10^8 copies/mL. This assay, still, requires more optimization to increase the sensitivity to 1,000 copies/ml [33].

A highly sensitive localized Surface plasmon Resonance immunosensor was developed by Lee, et al. for the detection of HIV-1 virus. In order to measure different concentrations of HIV-1 particles, they used various gold nanostructures modified with HIV-1 antibody fragments. A 200 fg/mL detection limit was observed. These measurements were based on the shift of longitudinal wavelength in the UV–Visible spectrum resulting from changes in the local refractive index due to specific antigen-antibody interactions [34].

Inci et al. also developed a quantification assay to monitor the HIV viral load by the use of nanoplasmonic platform for many HIV subtypes. This assay demonstrated a high sensitivity of upto 50 HIV copies/mL. Nanoplasmonic properties are used to monitor the captured viruses on the biosensor, and the platform reports a viral load for HIV-infected unidentified patient whole blood samples [35].

Another device has been used to quantify $CD4^+$ cells in patients that were infected with HIV in resource-limited sites. $CD4^+$ T cells were captured in microfluidic channel and then imaged using lens free shadow imaging system. The number of cells was then calculated by computer-aided software within a minute. This platform was established to work with HIV-positive patients in Tanzania, and compared with conventional methods of

cell counting [36].

Diao, et al. developed a highly sensitive SPR biosensor for the detection of HIV-related DNA. This device was based on ESDRs (entropy-driven strand displacement reactions) and DDTs (double-layer DNA tetrahedrons). ESDRs were utilized for the formation of ample dsDNA products. These dsDNA products bind to capture probes and then combine with DDTs nanostructure. Through this the SPR response was increased a lot. This biosensor is capable of detecting target DNA ranging from 1 pM to 150 nM with a LOD of 48 fM. It took only 60 minutes to complete the entire detection process [37], Figure 5.

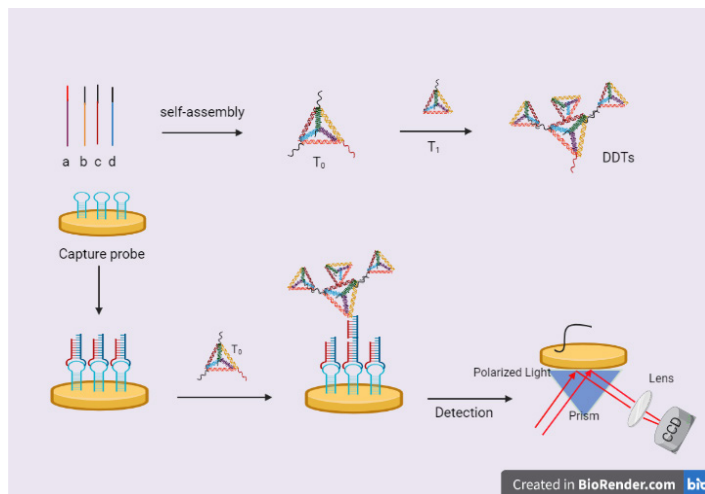


Figure 5: Schematic illustration of SPR biosensor for HIV detection and assembly of DDTs [37].

Piezoelectric biosensors for HIV

Piezoelectric biosensors include Quartz Crystal Microbalance (QCM)-based sensors and Surface Acoustic Wave (SAW) Sensor. Piezoelectric devices can record affinity interactions without the need to use any particular reagents [38].

Quartz crystal microbalance (QCM)-based sensors are mechanical sensors that comprise a piezoelectric material coated with a metal coating, such as gold or silver [39]. Molecularly Imprinted Polymers are specific artificial materials that can be used in place of antigens or antibodies as the biorecognition part in a biosensor [40]. In the following study, Lu, et al. established a quartz crystal microbalance platform that was treated with molecularly imprinting polymer to imitate biomolecular confirmation of Glycoprotein41 (gp41) epitope of HIV-1. Gp41 is required for the fusion of membrane between infected cells and virus. Dopamine has analog residues with gp41 and was used as a functional monomer. Then, gp41 proteins were coated on to the surfaces of these sensors. The results revealed that molecularly imprinted film binds to Gp41 protein selectively and the detection limit was 2 ng/mL [41].

Ly, et al. developed an assay proposing the recognition of HIV-1 antigen by the use of Quartz Crystal Microbalance surface treated with streptavidin and 11-mercaptoundecanoic acid (MUA). Streptavidin-functionalized gold nanoparticles were used for signal enhancement. Due to this amplification of signal detection of low concentration of HIV-1 antigen was possible [42].

Sheikh, et al. developed a piezoelectric acoustic immunosensor for detection of HIV-2 by the use of HIV-2 immunodominant epitope-functionalized self-assembled monolayer-coated quartz wafer. The results showed that the immunosensor is effective in detecting and differentiating HIV-2 from HIV-1 monoclonal antibodies with good selectivity. Equimolar serum solutions of HIV-2 or HIV-1 were used for the evaluation of specific and non-specific interactions by electromagnetic piezoelectric acoustic sensor (EMPAS) [43].

The following table 1 summarizes the key features of various HIV biosensors.

Biosensor	Biorecognition element	Lowest limit of detection	Reference
Amperometric	HIV P24 Antibodies	0.008 ng/mL	[26]
Electrochemoluminescence	HIV Aptamer	0.3 fM	[27]
Impedance	Anti-Gp 120 antibodies	Between 100 Hz and 1 MHz	[28]
Graphene-Au nanoclusters Based	Cytosine-rich capture probes	30 aM	[29]
DPV based	HIV gene sequence	8.7 fM	[30]
PAN/GN nanocomposite based	HIV-1 gene	1.0×10^{-16} M	[31]
TSDR and Cruciform DNA crystal based	HIV DNA sequences	0.21 pM	[44]
Label-free multiplexed biosensor	Hairpin DNA probes (HIV-1 and HIV-2 both)	0.1 nM	[45]
Carbon nanotubes based MIP electrochemical sensor	HIV-p24 antigen	0.083 pg cm ⁻³	[46]
Anti-Tat RNA aptamer based	HIV-1 Tat protein	1:00 PM	[47]
Photonic crystal biosensor	Glycoprotein 120 antibody	10 ⁵ copies/mL	[33]
SPR Immunosensor	HIV-1 Antibody Fragments	200 fg/mL	[34]
SPR Biosensor	Hairpin Probes	48 fM	[35]
Nanoplasmonic Platform	Polyclonal anti-gp120 antibodies	50 HIV copies/mL	[36]
ESDRs/DDTs based SPR biosensor	HIV-related DNA sequence	48 fM	[37]
SERS based Lateral Flow Biosensor	HIV-1 DNA sequence	0.24 pg/mL	[48]
Hybridization chain reaction (HCR) based chemiluminescence biosensor	HIV-1	5.0 fM	[49]
SERS based Sub-attomolar Sensor	HIV-1 DNA	10 ⁻¹⁹ M	[50]
Zeolitic imidazolate framework-based fluorescence biosensor	HIV-1 DNA	1.2 nmol L ⁻¹	[51]
MIP coated QCM Sensor	Synthetic Gp41 epitope	2 ng/mL	[41]
Quartz Crystal Microbalance based Biosensor	HIV P24 Antibody	1 ng/ml	[42]

Table 1: Key features of various HIV biosensors.

Conclusion

Being fast, handheld and easy to use makes biosensors ideal for point-of-care applications. Diagnostic tests usually require large equipment, advanced laboratory facilities, trained staff and a lot of time. Moreover, early detection of disease leads to better and

successful treatment measures. That is why countries that lack these state of the art facilities suffer in terms of poor health and are most of the times facing different emerging infectious diseases like HIV. Hence, the focus in diagnostic research has shifted towards biosensing strategies over the last two decades.

Novel biosensing systems such as paper-based portable platforms are being used for the monitoring of target biomolecules. They are affordable, abundant and deliver results in a matter of minutes. In the future paper-based biosensors will be able to detect multiple pathogens at the same time [52].

The use of smartphone and mobile applications has also been incorporated with paper-based platforms because of high processing capabilities, inexpensive and portable nature [53]. Similarly wearable or implantable sensors are also expected to be of great use in resource-constrained settings for monitoring a patient's health status and are made with flexible materials to be used as skin patches [54]. Moreover many types of CRISPR/Cas based biosensing technologies are being developed for the detection of SARS-CoV-2 [55]. But more research and examination is required to unifying the compartmentalization of such procedures onto a single device. Although these technologies are still in their early development phase, they are expected to be great contributors in eradication of infectious agents like HIV as well.

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