Talanta 206 (2020) 120201

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

HIV biosensors for early diagnosis of infection: The intertwine of nanotechnology with sensing strategies

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ARTICLE INFO

Keywords:
Biosensing assays
Early detection of HIV
Nanomaterials

ABSTRACT

Human immunodeficiency virus (HIV) is a lentivirus that leads to acquired immunodeficiency syndrome (AIDS). With increasing awareness of AIDS emerging as a global public health threat, different HIV testing kits have been developed to detect antibodies (Ab) directed toward different parts of HIV. A great limitation of these tests is that they can not detect HIV antibodies during early virus infection. Therefore, to overcome this challenge, a wide range of biosensors have been developed for early diagnosis of HIV infection. A significant amount of these studies have been focused on the application of nanomaterials for improving the sensitivity and accuracy of the sensing methods. Following an introduction into this field, a first section of this review covers the synthesis and applicability of such nanomaterials as metal nanoparticles (NPs), quantum dots (QDs), carbon-based nanomaterials and metal nanoclusters (NCs). A second larger section covers the latest developments concerning nanomaterial-based biosensors for HIV diagnosis, with paying a special attention to the determination of $CD4^+$ cells as a hall mark of HIV infection, HIV gene, HIV p24 core protein, HIV p17 peptide, HIV-1 virus-like particles (VLPs) and HIV related enzymes, particularly those that are passed on from the virus to the CD^{4+} T lymphocytes and are necessary for viral reproduction within the host cell. These studies are described in detail along with their diverse principles/mechanisms (e.g. electrochemistry, fluorescence, electromagnetic-piezoelectric, surface plasmon resonance (SPR), surface enhanced Raman spectroscopy (SERS) and colorimetry). Despite the significant progress in HIV biosensing in the last years, there is a great need for the development of point-of-care (POC) technologies which are affordable, robust, easy to use, portable, and possessing sufficient quantitative accuracy to enable clinical decision making. In the final section, the focus is on the portable sensing devices as a new standard of POC and personalized diagnostics.

1. Introduction

Acquired immunodeficiency syndrome (AIDS) was initially reported by the US Center for Diseases Control in 1981 followed by the identification of human immunodeficiency virus (HIV) as the cause of the disease in 1983 [1]. AIDS, as one of the great challenges in this century, is rapidly rising. According to the obtained statistics, there were approximately 36.9 million people living with HIV at the end of 2017 with a global HIV prevalence of 0.8% among adults [2]. More specifically, an estimated 940,000 people died from HIV-related causes globally in 2017 (see Table 1 for the number of AIDS-related deaths). The risks posed by AIDS syndrome have been recognised as critical threats to mankind. Therefore, the early diagnosis of HIV infection is of great importance for all scientists around the world.

There is currently a remarkable demand to identify HIV in a fast, selective and accurate way. HIV infection is often diagnosed through enzyme-linked immunosorbent assay (ELISA), which detects the presence or absence of HIV antibodies (such as IgM/IgG Abs) [3]. This test analyzes blood or saliva for antibodies to the virus. Since the HIV antibody test was first made widely available in Canada in 1985 [4], the HIV testing has been a policy challenge for health authorities. Although this test is very sensitive, it requires a “window period” after infection with HIV [5]. The window period can vary from a few weeks to a few months after infection. In fact, the human immune system takes some time to produce antibodies to HIV; this does not happen immediately upon infection. In addition, ELISA involves several incubation and washing steps followed by spectrophotometric detection using a chromogenic substrate. It is recognised, therefore, that HIV testing should be promoted and conducted in methods that detect HIV during early virus infection.

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https://doi.org/10.1016/j.talanta.2019.120201
Received 26 June 2019; Received in revised form 30 July 2019; Accepted 30 July 2019
Available online 31 July 2019
0039-9140/

Table 1
The worldwide statistics for HIV cases and HIV death toll.

Country	People living with HIV/AIDS	HIV/AIDS deaths
South Africa	7,100,000	110,000
Nigeria	3,200,000	160,000
India	2,100,000	62,000
Kenya	1,600,000	36,000
Zimbabwe	1,300,000	30,000
Zambia	1,200,000	21,000
Brazil	830,000	14,000
Thailand	450,000	16,000
Ghana	290,000	15,000
Ukraine	240,000	8500
Vietnam	250,000	8000
Mexico	220,000	4200
France	180,000	1000
Pakistan	130,000	5500
Colombia	120,000	2800
Argentina	120,000	2400
Iran	66,000	4000
Sudan	56,000	3000
Senegal	41,000	1900
Nepal	32,000	1700
Australia	25,000	500
Afghanistan	7500	500
Egypt	11,000	500
Saudi Arabia	8200	500
Netherlands	23,000	200
Cuba	25,000	200
Czech Republic	3400	100

Information in this table is accurate as of January 1, 2018; https://www.indexmundi.com.

More than 30 years after the HIV/AIDS epidemy, many experts have suggested ultrasensitive biosensing methods for the fast determination of $CD4^+$ cells, HIV genes and HIV biomarkers [5]. In contrast to the immunoassays in which the antibodies need a long period to generate, the detection of HIV related proteins or genes presents an alternative strategy for early diagnosis of the infection. Furthermore, the biosensing strategies do not suffer from the other disadvantages of antibody-based HIV detection methodologies such as high cost and complexity.

In recent years, several biosensors have been designed for detection of HIV infection [5]. Although the biosensors are suitable alternatives for ELISA kits, most of them suffer some limitations such as low sensitivity. In addition, the fabrication of multiplexed biosensors for simultaneous detection of different analytes has still remained a major challenge in the bioanalytical frontier [6]. Fortunately, nanotechnology can overcome the current challenges in designing sensors and enhance their sensitivity. Nanomaterials offer simple conjugation of bioreceptors on their surfaces to facilitate the targeted sensing. Compared to classic sensors for the single analyte assay, sensor arrays based on the multifunctional nanomaterials can provide the ability for multiplexed analytes detection. More importantly, the ease of mass transfer at very short distances makes the response of nanomaterial-based sensors rapid, which is helpful in the development of fast-response sensors [7]. Currently, the sensing methods are benefiting from nanotechnology and targeting properties of the recognition elements in order to design new biosensors that simultaneously detect target species with enhanced sensitivity and selectivity together with high speed.

Recently, nanoscale materials have attracted considerable attention because of their unique properties that differ substantially from those of the corresponding atoms and bulk materials. In general, the nanomaterials have superior properties compared with traditional bulk materials because of their significantly enlarged surface-to-volume ratio, which resulted in more active sites [8]. By engineering the size and morphology, some robust nanomaterials with excellent electrochemical and luminescent properties can be obtained. It is well established that the size, composition and surface properties of nanostructures play a significant role in the sensing procedure. Although enormous efforts have been put on the development of nanomaterial-based HIV biosensing devices, most of them are yet under diverse clinical trial stages.

A recent review paper [9] has summarized major studies of molecular tools used for the HIV detection including HIV quantitative nucleic assays and qualitative nucleic acid assays. The presented molecular technologies were mainly based on the polymerase chain reaction (PCR), transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and branched chain DNA methods and point-of-care (POC) testing. Here, we offer a comprehensive survey on the advances, challenges and opportunities of sensing technologies for determination of HIV markers such as HIV type 1 or HIV type 2 antibodies, viral DNA (RNA), viral P24, p17, HIV-related enzymes and $CD4^+$ T lymphocytes. Moreover, various sensing strategies including nanoscaled electrochemical detection, nanomaterial-amplified optical assays, electromagnetic methods as well as surface plasmon resonance (SPR) and surface-enhanced Raman spectroscopy (SERS) for early diagnosis of HIV infection, are also thoroughly reviewed and explained in detail. At last, this review offers prospects for the future research and development needed for POC sensing technology, especially connected with smartphones for in situ and real-time detection of HIV.

2. Nanomaterials: advanced structures explored for HIV detection

Nanomaterial is the term related to nanotechnology due to it obeys all principles of nanotechnology. The International Organization for Standardization (ISO) has described nanomaterials as a “material with any external nanoscale dimension or internal nanoscale surface structure” [10]. In addition, nanoscale can be defined as—the dimension ranges from 1 nm to 100 nm approximately. Nanosheets, nanotubes, nanorods, nanofibers, nanoplates, nanowires, nanobelts, nanodots and other related terms have been defined based on this ISO definition [6]. Many types of nanomaterials have been reported and other varieties are predicted to appear in the future. Therefore, the need for their classification has ripened. The first idea for the classification of nanomaterials was given by Gleiter et al. [11]. They classified the nanomaterials depending on their crystalline forms and chemical composition. However, the Gleiter scheme was not fully complete because the dimensionality of the nanomaterials was not considered. In 2007, Pokropivny and Skorokhod [12] made a new scheme of classification for nanomaterials which included the recently developed composites such as 0D, 1D, 2D and 3D nanomaterials (see Fig. 1). This classification is highly dependent on the electron movement along the dimensions in the nanomaterials.

The rapid pace of advancements in the field of nanotechnology has driven innovations in all the disciplines of science including biosensors where cutting-edge research is carried out for the development of disease diagnosis. The nanoscale materials are adding a value to the existing sensors, thereby opening new opportunities in the sensing area. They enhance loading of recognition elements on the platforms, speed of response, signal amplification, sensitivity, detection range, selectivity, interference resistance, specificity, stability and cost-effectiveness. Importantly, multifunctional nanomaterials play a critical role in the development of multi-modality sensing tools and point-of-care devices. Considering the great role of nanostructures in the biosensing assays, the more recent nanomaterials for the HIV sensing have comprehensively reviewed in this section.

2.1. Metal and metal oxide nanoparticles

In 1857, Michael Faraday reported the synthesis of the colloidal gold nanoparticles (AuNPs) [13], which was the first scientific description for preparation of nanomaterials. He also revealed that the optical characteristics of Au colloids are dissimilar compared to their respective bulk counterpart. This was probably one of the earlier reports where the quantum size effect was observed and described. Later,

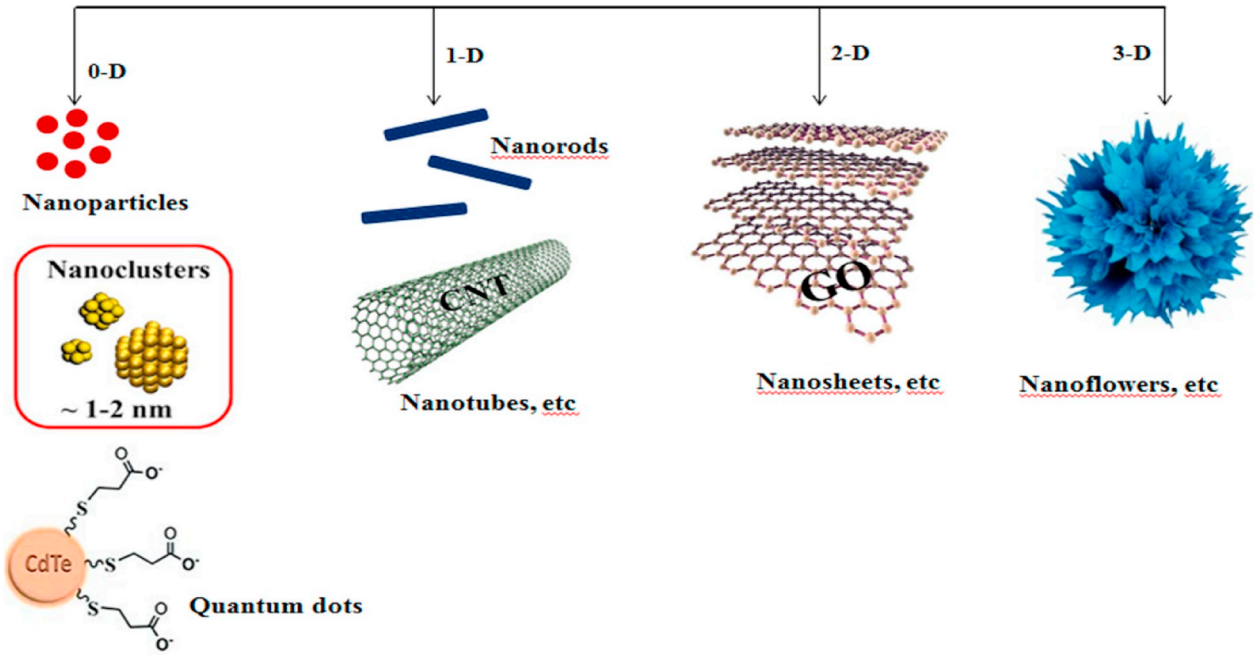

Fig. 1. Diagram of classification and examples of nanomaterials with 0D, 1D, 2D and 3D structures.

Mie (1908) explained the reason behind the specific colors of metal colloids [14]. Today, the manufactured nanoparticles can significantly improve the characteristics of bulk materials, in terms of strength, conductivity, durability, and lightness and can function as reinforcing materials for construction or sensing components for safety.

Among these nanoparticles, plasmonic NPs or nanosized noble metals (Au and Ag) have gained particular interest from various scientists and engineers because of their unique physical and chemical properties [15]. They have been widely applied in the biomedical fields for diagnostics, imaging and therapeutics. Currently, a popular area in nanomedicine is the implementation of plasmonic NPs for the infection detection, attributed to the intriguing their optical properties. The surface plasmon resonance, a unique phenomenon to plasmonic noble metal NPs, leads to strong electromagnetic fields on the particle surface and consequently enhances all the radiative properties such as absorption and scattering.

AuNPs are one of the most extensively studied nanoparticles in nanoscience and nanotechnology when compared to other metal-based nanomaterials. Their highly favorable properties, including a large surface area-to-volume ratio, biocompatibility, unique optical and electronic properties, and easy surface modification, have resulted in an intensive focus on AuNPs from both academia and industry [16]. In addition, AuNPs can be widely used to adsorb biomolecules such as proteins, antibodies, and aptamers onto their surfaces [17]. Much effort has been devoted to tailoring the optical and electrical properties of AuNPs for specific biosensing applications. The researchers have been mainly focused on four different plasmonic biosensing methods, including localized surface plasmon resonance (LSPR), SERS, fluorescence enhancement, and quenching caused by plasmon and colorimetry changes based on the coupling of AuNPs [15]. Briefly, the localization of the electromagnetic field results in the amplification of SPR signals and SERS. The fluorescent signals can also be quenched or enhanced due to the energy transfer between the AuNP and fluorophore [15].

AgNPs are capable of facilitating electron transfer from the reaction center to the electrode surface, which makes them capable of higher intensity electron transfer. AgNPs have few desirable properties such as ease-of-functionalization, good biocompatibility, ease of immobilization of biomolecules and ability to increase the electrochemiluminescence (ECL) intensity of luminophores. These facts have led AgNPs to be incorporated in ECL nanobiosensor fabrication [18]. In addition, nanosilver substrate is one of the most effective metal nanostructures for SERS. The SERS using AgNPs have been used to obtain reproducible intense signals for detection of molecules in aqueous solutions [19]. These nanoparticles are characterized by an intense absorption band in the UV–Vis region, which is not present in the spectrum of the bulk metal. Overall, the AgNPs have been exploited for the development of protocols involving metal enhanced fuorescence-based detection, SERS and, in combination with Au, to form bimetallic NPs which can either be alloys or core shell nanoparticles [20].

Recent advances in nanotechnology have revealed new possibilities for the development of biosensors with improved performance based on the unique properties of metal oxide NPs. Iron oxide NPs consisted of maghemite (γ-Fe_2O_3), hematite (α-Fe_2O_3) and magnetite (Fe_3O_4) with diameters ranging from 1 to 100 nm have found broad applications in the magnetic separation, biosensing and drug-delivery [21,22]. They are an emerging focus in the development and fabrication of sensors and biosensors, due to their unique properties, such as superparamagnetism, large surface-to-volume ratio, greater surface area, and easy separation methodology [21]. Magnetic NPs possess large surface areas and high mass transference which enhance the sensitivity and stability in the fabrication of biosensors and other detection systems in clinical, food and environmental applications.

2.2. Heavy metal quantum dots

Among the most interesting and promising nanomaterials are colloidal semiconducting quantum dots (QDs). These nanostructures have already found several commercial applications in various bio-applications. The main reasons for their still growing success include broad absorption band (several hundreds of nm), narrow emission band (below 40 nm), high quantum yield (QY, up to 95%), possibility of emission band tuning over a wide range of wavelengths (350–2000 nm) and high resistivity of optical properties on external physico-chemical conditions, such as solution pH, temperature and power of the excitation beam [23]. QDs have a high surface to volume ratio, which can be controlled not only by QDs size but also by the shape of nanostructures. This high surface area equips them with much more functional groups, compared to organic compounds, which makes the QDs much more

reactive and thus more effective in biological sensing devices [24]. Despite the toxicity of heavy metal QDs, this limitation becomes a much less serious problem when the QDs are used for external sensing or for some in vitro applications.

2.3. Carbon-based nanomaterials

With the development of carbon nanomaterials in recent years, there has been an explosion of interests in their application to development of new biosensors. It is believed that employing carbon nanomaterials as sensor components can make sensors more reliable, accurate and fast, due to their remarkable properties.

Carbon nanotubes (CNTs) are elongated cylindrical structures with diameters of 1 to several dozens of nanometers and lengths of up to several microns consisting of one or several hexagonal graphite planes rolled in tubes. Their surface consists of regular hexagonal carbon cycles (hexagons). Depending on nanotube synthesis conditions, one (SWCNT) or multilayered (MWCNT) tubulenes with open or closed terminations may form. Today, CNTs are employed for many applications, encompassing the field of reinforcement electronics, optoelectronics, sensors, batteries and supercapacitors [25].

Graphene, a single atom thick layer of two-dimensional closely packed honeycomb carbon lattice, and its derivatives have attracted much attention in the biomedical fields, mainly due to its unique physicochemical properties. The valuable physicochemical properties, including high surface area, excellent electrical conductivity, remarkable biocompatibility and ease of surface functionalization have shown great potentials in the applications of graphene-based bioelectronics devices, including electrochemical biosensors for the analysis of biomarkers [26,27]. Furthermore, graphene can be a promising candidate to make up the deficiency of a conventional metal SERS substrate [28]. Graphene, as a new member of SERS-active materials, would also play a vital role in boosting the practical application of the SERS technique in the near future.

Carbon dots (C-dots, CDs) or carbon quantum dots (CQDs) were first discovered in 2004 during single-walled carbon nanotubes purification [29]. They can be described as quasi-spherical particles with sizes below 10 nm. In the last decade, CDs have attracted a considerable amount of attention from the scientific community as a low cost and biocompatible alternative to semiconductor quantum dots. In particular, doped CDs have excellent fluorescent properties that have been successfully utilized for numerous applications such as chemo/biosensors, bioimaging, drug delivery, catalysis, optoelectronic devices and etc [30]. As is well known, CDs with fine performance, especially high fluorescence QY, could maximize their desired applications in those areas. However, efficient methods for the synthesis of CDs with high QY are still remaining as a challenge.

Graphene quantum dots (GQDs) as the fluorescence probes are a kind of 0D materials with characteristics derived from both graphene and carbon dots (CDs). Combining the structure of graphene with the quantum confinement and edge effects of CDs, GQDs possess unique properties [30].

2.4. Metal nanoclusters

Biological macromolecules (protein, DNA) coated metal nanoclusters (NCs) are used as excellent scaffolds for the development of chemical and biological sensors due to their outstanding physical and chemical properties [31]. These metal nanoclusters comprising a few to tens of atoms, as a new class of nano-fluorophores, are biocompatible. They display strong fluorescence emission due to the quantum-confinement effect [32] when their sizes are reduced to around 2 nm or less, and the band structures transfer to discontinuous and discrete energy levels, which are similar to the energy levels of molecules [33].

To date, extensive research on fluorescent metal nanoclusters has mainly focused on the noble metal nanoclusters (especially Au and Ag NCs). Fluorescent Ag and Au NCs have attracted special attention due to their merits of being economical, green, and easy to fabricate. Besides the facile and low-cost synthesis, the greatest advantage of Au and Ag NCs compared to semiconductor quantum dots and organic dyes is that one can obtain a fluorescent probe with a specific binding ligand through a single step. For example, Shamsipur et al. presented an aptamer-Ag NCs for specific detection of cytochrome *c* [34]. However, noble metal NCs have become a research hotspot in the biomedical analysis area, the studies on tiny Cu NCs are still deficient owing to their inherent instability.

3. HIV: possible biosensing strategies

While there have been remarkable efforts to develop new methods for detecting and treating HIV, it has been challenging to translate them into sensitivity-enhanced strategies. Currently, electrical or optical sensing technologies are used to report binding and recognition events occurring on a sensing surface, including antigen-antibody, target-aptamer, nucleic acid hybridization and enzyme-cofactor coupling. However, most of research efforts have been devoted to developing POC diagnostics or resource-limited settings that can monitor HIV viral load with high sensitivity by leveraging nano-scale technologies. In this section, we discuss the current challenges in HIV diagnosis and present emerging technologies that aim to solve these challenges using novel nanoscale solutions.

3.1. HIV/AIDS-symptoms and causes

HIV is roughly spherical with a diameter of about 120 nm, around 60 times smaller than a red blood cell (see Fig. 2). It is composed of two copies of positive-sense single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2000 copies of the viral protein p24. The single-stranded RNA is tightly bound to enzymes needed for the development of the virions such as reverse transcriptase, proteases and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle. This is, in turn, surrounded by the viral envelope, that is composed of a lipid bilayer taken from the membrane of a human host cell when the newly formed virus particle buds from the cell.

HIV is a deadly virus that attacks the body's immune system, specifically the CD4 cells which are a type of T cells. These are white blood cells that move around the body, detecting faults and anomalies in cells as well as infections. When HIV targets and infiltrates these cells, it reduces the body's ability to combat other diseases. This increases the risk and impact of opportunistic infections and cancers. Although the symptoms of HIV can vary between individuals, the first signs of infection generally appear within the first 1–2 months. Many, but not all, people will experience severe flu-like symptoms which is body's natural response to the virus. This is called the 'seroconversion' period. Many people experience digestive system problems or swollen lymph nodes as a symptom of the early stages of HIV. Skin rashes can occur early or late in the course of HIV seroconversion. In some cases, the rash can appear similar to boils with itchy, pink breakouts. However, a person can carry HIV without experiencing symptoms for a long time. Without treatment, HIV infection is likely to develop into AIDS as the immune system gradually wears down. AIDS is the most advanced stage of HIV infection.

There are two major types of the human immunodeficiency virus. HIV-1, which was discovered first, is the most widespread type worldwide. According to the HIV awareness charity Avert, around 95% of people living with HIV have HIV-1. HIV-1 is more virulent and transmittable than HIV-2. HIV-2 is more than 55% genetically different from HIV-1. HIV-2 is mainly present in West Africa, but it is slowly starting to appear in other regions, including the United States, Europe, and India. Both types of HIV weaken the immune system, but HIV-2 tends to develop more slowly and is less easy for people to transmit,

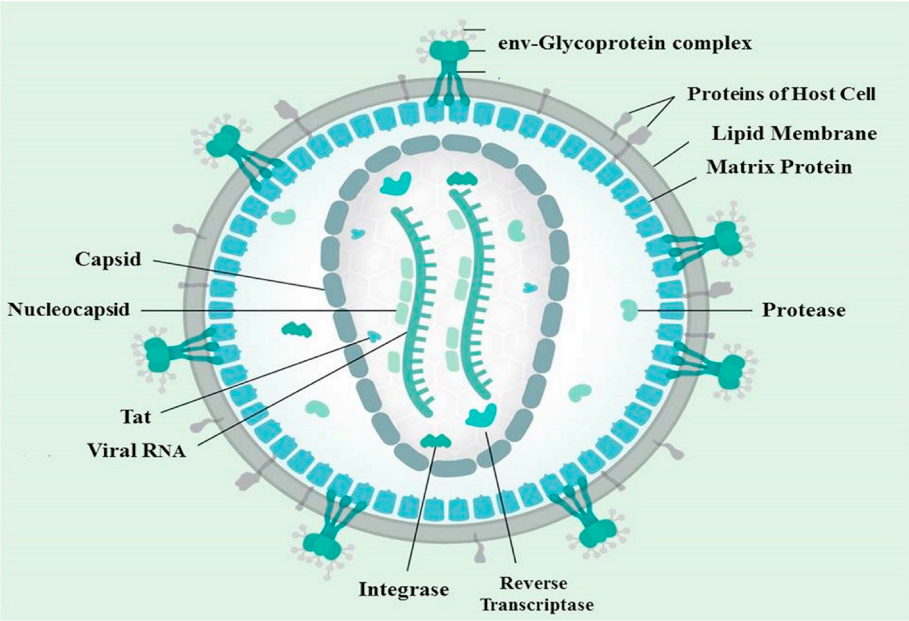

Fig. 2. Diagram of the HIV virion.

compared to HIV-1. Due to this genetic difference, HIV-1 and HIV-2 antigens are distinct enough. The genetic differences between the two viruses mean that there are some differences in how healthcare providers diagnose and treat HIV-1 and HIV-2.

HIV can be transmitted via the exchange of a variety of body fluids from infected individuals, such as blood, breast milk, semen and vaginal secretions. Individuals cannot become infected through ordinary day-to-day contacts such as kissing, hugging, shaking hands, or sharing personal objects, food or water. The risk of HIV transmitting through blood transfusions is extremely low in countries that have effective screening procedures in place for blood donations.

3.2. Viral markers in HIV infection and AIDS

Viral and immune markers are used for monitoring either progression of HIV disease or response to antiviral therapy. Ideal properties of viral markers are that they are present in all HIV-infected persons at all stages of disease, that they are related to disease pathogenesis, that they can be easily quantitated, that this quantitation correlates rapidly and predictably with both disease stage and response to antivirals, and that they can be developed into rapid, reproducible automated tests. The available viral markers include HIV p24 antigen (HIV p24 core protein, after acid glycine dissociation), *anti*-p24 antibody titres, HIV p17 peptide, HIV-1 Tat protein, $CD4^+$ cells, HIV gene, HIV-1 virus-like particles (VLPs), HIV related enzymes and quantitative DNA and RNA polymerase chain reaction performed on cells and plasma, and HIV isolate phenotype [35–40]. At present, the majority of the developed methods for HIV diagnosis are based on the detection of the presence of its components (i.e., specific nucleic acid sequences or antigens or measurement of the activity of enzymes) instead of the measurement of antibodies made in patient's body against HIV or direct molecular recognition of HIV.

3.2.1. $CD4^+$ cells

$CD4^+$ T cells are white blood cells that are specifically targeted and destroyed by HIV. Even if a person has no symptoms, HIV infection progresses to AIDS when $CD4^+$ T cell count dips below 200. In fact, the hallmark of AIDS pathogenesis is a progressive depletion of $CD4^+$ T-cell populations in close association with progressive impairment of cellular immunity.

3.2.2. Viral RNA

HIV-1 RNA has become an important standard for monitoring antiretroviral therapies. It has been shown that the concentration of HIV RNA in plasma is predictive of $CD4^+$ T cell decline, progression to clinical AIDS and survival [38]. In fact, the measurement of HIV-1 RNA plasma levels (viral load) provides quantitative detection of viremia and is used in conjunction with $CD4^+$ T-cell counts.

3.2.3. HIV-1 tat protein

The HIV-1 Tat is a key activator of HIV-1 transcription. It is one of the first proteins to be expressed after infection occurs. Unlike typical transcription factors that are DNA binding proteins, Tat is an RNA binding protein that recognizes a specific sequence called as TAR (Transactivator Response Element), from the HIV-1 RNA molecule. It is generally acknowledged that the Tat protein has a pivotal role in HIV-1 replication because it stimulates transcription from the viral long terminal repeat (LTR) promoter by binding to the TAR hairpin in the nascent RNA transcript [41].

3.2.4. HIV core protein p24

The p24 antigen testing is similarly sensitive and specific in diagnosing pediatric HIV infection, in predicting $CD4^+$ T cell decline and clinical progression at early and late stage of infection, and suitable for antiretroviral treatment monitoring in both adults and children. Notably, p24 antigen was measurable even in patients with stably suppressed viremia, and its concentrations were correlated negatively with the concentrations of $CD4^+$ T cells and positively with the concentrations of activated $CD4^+$ T cell subsets. The HIV core protein p24 is an excellent marker of HIV expression and disease activity and can be used in the same fields of application as HIV RNA is used [38]. It is a viral protein that makes up most of viruses. This protein is produced 2–3 weeks after infection.

With the advent of nucleic acid amplification methods for monitoring HIV-1, the measurement of HIV-1 p24 antigen has a much more limited role than it once did. Although antigen detection is a less expensive alternative to viral RNA detection for this purpose, the viral RNA is significantly more sensitive than the detection of p24 antigenemia [42].

3.2.5. HIV-1 matrix protein p17

Fragments of HIV p17 protein ranging in length from about 12 to about 40 amino acids are used to form diagnostics and vaccines for detection or treatment of AIDS. The HIV-1 matrix protein p17 is a structural protein critically involved in most stages of the life cycle of the retrovirus. It participates in the early stages of virus replication as well as in RNA targeting to the plasma membrane, incorporation of the envelope into virions and particle assembly. Moreover, p17 is involved in viral RNA binding and transport to the plasma membrane, in incorporation with the HIV-1 envelope into virions, as well as in particle assembly. It has been reported that p17 can be the target of neutralizing antibodies against HIV-1 and that high levels of p17 antibodies are correlated with slower progression of AIDS [43].

3.2.6. Viral infectivity factor

The viral infectivity factor (Vif), a 23 kDa protein, is mainly found in the cytoplasm of HIV-1 infected cells. Vif is considered as a phosphoprotein and phosphorylation seemed to be required for viral infectivity. It is also identified as a potential target in antiviral therapy. Targeting Vif has been suggested as a strategy for future HIV drug therapies.

3.2.7. HIV enzymes

After HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase and protease are injected into the cell. The retroviral RNA genome encodes for three enzymes essential for virus replication: (i) the viral protease that converts the immature virion into a mature virus through the cleavage of precursor polypeptides; (ii) the reverse transcriptase, responsible for the conversion of the single-stranded genomic RNA into double-stranded proviral DNA; and (iii) the integrase that inserts the proviral DNA into the host cell genome. All of these are important targets for therapeutic intervention [44].

3.3. Nanomaterial-assisted HIV sensing strategies

In this section, we introduce emerging technologies, categorized by their transducing methods, which are being used to quantitatively detect HIV via direct (*i.e.*, the capture of intact virus), indirect (*i.e.*, the capture of host antibodies to the virus) with emphasized on the nanotechnology.

3.3.1. Direct detection

HIV is present as both free virus particles and virus within infected immune cells. Normally, all of the existing viruses require a host to propagate their lives and many are related to the human pathogens. Therefore, rapid detection of HIV has emerged due to its impact on the human health and quality of life.

Virus-like particles (VLPs) provide promising platforms for the development of biosensing vessels for the virus detection, because they have the advantages of viruses without the risk of disease. VLPs represent a unique class of particles which resemble the structure of authentic viruses but lack core viral genetic materials and, thus, have no infectivity. They can be naturally occurring or synthesized through the individual expression of viral structural proteins, which can then self assembled into a virus-like structure [45]. Due to the special feature of VLPs, they have been widely used for different functions such as gene therapy, vaccination, nanotechnology and diagnosis [46]. For example, the identification and quantification of HIV-1 VLPs have been provided a novel methodology for the detection of HIV.

In recent years, imaging-based techniques such as transmission electron microscopy (TEM) [47], advanced amplitude modulation-frequency modulation (AM-FM) viscoelastic mapping mode [45] and fluorescence microscopy [48] has opened a new window for the identification of HIV-based VLPs. However, these techniques are somewhat low-throughput, labor-intensive, and require high-level technical expertise to operate the costly associated equipment. Additionally, special treatment of samples is required, which sometimes results in inaccurate measurements due to aggregation and deformation of virus particles [49].

The development of a rapid, real-time, label-free and sensitive system to detect VLPs must address the problems and limitations associated with the aforementioned technologies. One promising approach is the use of biosensing strategies based on the nanomaterials. For example, Lee et al. [50] reported an electrochemical method to detect direct electron transfer signal from the HIV-1 virus. In this strategy, AuNPs were electrodeposited on the indium tin oxide coated glass (ITO) electrode to provide better electron-transfer kinetics and higher background charging current. On the AuNPs modified ITO electrode, antibody fragment was immobilized by self-assembly method with gold–thiol interaction and different concentrations of HIV-1 virus-like particles (VLPs) were applied for the direct determination. The described sensor successfully detected VLP from 600 fg mL^{-1} to 375 pg mL^{-1}. In a similar study, a highly sensitive label-free immunosensor for the detection of HIV-1 was developed based on LSPR method by Lee and co-workers [51]. Uniform nanopattern of circular Au-dots (10–20 nm) was fabricated on ITO coated glass substrate by a simple electrochemical deposition method. The surface of Au nanopattern was modified with HIV-1 neutralizing gp120 monoclonal antibody fragments. The modified substrate was then employed to measure various concentrations of VLPs quantitatively based on the shift of longitudinal wavelength in the UV–Vis spectrum which results from the changes of local refractive index induced by specific antigen-antibody recognition events. The limit of detection (LOD) and dynamic linear range (DLR) of the HIV-1 particles were estimated to be 200 fg mL^{-1} and 200 fg mL^{-1}-125 pg mL^{-1}, respectively. Although, the described strategies demonstrate great capabilities of sensing HIV-VLPs, there are not more studies in this field.

3.3.2. Indirect detection

HIV diagnosis is routinely performed using indirect methods. In this case, clinical samples are evaluated to determine virus components, such as proteins or nucleic acids, are present. In fact, most of HIV biosensing methods are based on the indirect assays for determination of HIV genes or virus-related proteins. These biosensing strategies are reviewed as follows:

3.3.2.1. Electrochemical biosensors. Since the development of electrochemical sensor by Clark and Lyons in 1962 [52], much attention has been paid to generation of more efficient biosensors. Initial studies were focused on electrochemical sensing of simple metabolites such as urea, lactate or glucose, but now it has been extended to complex molecules such as proteins and viruses. This is due to their close link to developments in low-cost production of microelectronic circuits and their easy interface with normal electronic read-out and processing. Other inherent advantages of electrochemical biosensors are their robustness, easy miniaturization, excellent detection limits, small analyte volumes, portable, and ability to be used in turbid biofluids with optically absorbing and fluorescing compounds [53].

Currently, HIV components can be electrochemically detected with real-time once the patient was infected. In this field, an extensive effort is devoted to design novel electrochemical HIV biosensor arrays to improve the sensitivity, to reduce the cost and to simplify the assay steps. These include the use of different electrode materials and electrode configurations, development of efficient immobilization methods, exploitation of novel redox probe and label-free strategies. Table 2 summarizes the characteristics of different nanomaterial-based electrochemical sensors considering the electrode configurations [54–86]. As seen, these sensors demonstrated excellent sensitivity, specificity, reusability, and reproducibility. The specificity in these potent sensors was achieved by conjugation of specific biomolecules such as aptamers, antibodies, and enzymes.

Table 2
A list of nanomaterials-based biosensors for electrochemical detection of HIV biomarkers.

HIV makers	Type of affinity assay	Determination method	LOD	DLR	References
HIV-2 Ab	CVD-grown graphene sheets-based FET	–	12 pM	0.05–25 nM	[54]
$CD4^+$ cells	Electrode pixels	EIS	–	–	[55]
HIV-1 Protease	GE/SWCNT/AuNPs/Fc-pepstatin conjugate	EIS	0.8 pM	–	[56]
	SPGE/SWCNT/AuNPs/Fc-pepstatin conjugate	DPV	–	–	[57]
HIV-1 reverse transcriptase	SPCE-AuNPs/Fc-LA	SWV	0.7 fM	0.9–427 fM	[58]
	GE/FC-labeled target peptide	SWV	50 pg mL^{-1}	100–1000 pg mL^{-1}	[59]
HIV-1 Tat protein	Diamond FET using RNA aptamers	–	–	–	[60]
P17	GE/Lignin/Peptide layer-by-layer	EIS	–	0.1–100 ng mL^{-1}	[61]
P24	GE/Mercapto succinic acid hydrazide copper (II)/HRP-Ab	DPV	0.2 μg L^{-1}	0.5–50 μg L^{-1} 50–200 μg L^{-1}	[62]
	GE/Polytyramine-AuNP/Ab	Capacitive flow injection	0.079 fg L^{-1}	2.4 fg L^{-1}–2.4 pg L^{-1}	[63]
	GE/AuNPs/CNT/AEP/Ab-biotin-streptavidin-HRP	CV	0.0064 ng mL^{-1}	0.01–60 ng mL^{-1}	[64]
	GCE/AuNPs/Ab_1/p24/Ab_2-HRP	DPV	0.008 ng mL^{-1}	0.01–100 ng mL^{-1}	[65]
	SPCE/Fe_3O_4@SiO_2/Ab_1/p24/Ab_2-HRP/AuNPs	DPV	0.0005 ng mL^{-1}	0.001–10 ng mL^{-1}	[66]
	GCE/AuNPs/Ab_1/p24/Ab_2/Au@RGO/Ru–SiO_2	ECL	1.0 pg mL^{-1}	1.0 pg mL^{-1}–10 ng mL^{-1}	[67]
	GCE/MWCNT/HRP/SiO_2/Chitosan/GA/Ab_1/p24/Ab_2-HRP-GO-Thi	DPV	0.15 pg mL^{-1}	0.5 pg mL^{-1}–8.5 ng mL^{-1}	[68]
	GCE/MWCNT/Chitosan/GA/p24/MIP (AAM/MBA/MBA/APS)	DPV	0.083 pg cm^{-3}	0.1 pg cm^{-3}–2.0 ng cm^{-3}	[69]
Gene	PtE/PPy/Ag–Au nanocomposite/pDNA	EIS	0.5 nM	1.0 nM–1.0 μM	[70]
	GE/Fe_3O_4-Streptavidin/Biotin-pDNA	Non-faradic impedance spectroscopy	160 pmol (in 20 μL sample)	–	[71]
	SPE/pDNA/MB	SWV	0.1 nM	20–100 nM	[72]
	SPE/Chitosan/Fe_3O_4/pDNA(GEM 91)/MB	SWV	50 pM	50–300 pM	[73]
	GCE/GO/PTCA/Au-IL/pDNA	EIS	34 pM	0.1 pM–1.0 μM	[74]
	GCE/GO/PTCA/pDNA	EIS	0.5 pM	1.0 pM–1.0 μM	[75]
	GCE-Ph-NH_2/GO/Immobilization sequence/pDNA	EIS	0.11 pM	1.0 pM–1.0 μM	[76]
	GE/pDNA/HIV DNA/NF-kB protein/HRP	Amperometry	7.05 pM	5.0–500.0 nM	[77]
	SPCE/GO-nafion/CA-pDNA(Molecular beacon)-CA	DPV	5 nM	40 nM–2.56 μM	[78]
	GCE/ERGO/pDNA	EIS	0.3 pM	1.0 pM–1.0 nM	[79]
	GCE/Graphene stabilized AuNC/pDNA-MB	DPV	30 aM	0.1 fM–100 nM	[80]
	GCE/Fe_3O_4/AuNPs/pDNA/HIV DNA/PV@CdS/AuNPs	SWV	0.2 fM	0.5 fM–500 pM	[81]
	GCE/RGO/Fe_3O_4/pDNA	DPV	2.0 aM	10 aM–1 nM	[82]
	GCE/Graphene-Nafion/pDNA	EIS	23 fM	0.1 pM–0.1 nM	[83]
	ITO/*o*-PD(Electropolymerization)/pDNA/HIV DNA-EuNCs-PAA	ECL	0.3 fM	3.0 fM–0.3 nM	[84]
	NiO-FET	–	0.3 aM	1.0 aM–10 nM	[85]
	GCE/ERGO/PABA/AuNPs/pDNA	EIS	37 aM	0.1 fM–10 nM	[86]

Ab: Antibody; GE: Gold electrode; GCE: Glassy carbon electrode; SPGE: Screen printed gold electrode; SPCE: Screen printed carbon electrode; ITO: Indium tin oxide; CVD: Chemical vapor deposition; FET: Field effect transistor; EIS: Electrochemical impedance spectroscopy; CV: Cyclic voltammetry; DPV: Differential pulse voltammetry; SWV: Square wave voltammetry; ECL: Electrochemiluminescence; SWCNT: Single-walled carbon nanotube; AuNPs: Gold nanoparticles; Fc: Ferrocene; Fc-LA: Ferrocene-labeled lipoic acid; RNA: Ribonucleic acid; HRP: Horseradish peroxidase; AEP: Acetone-extracted propolis; RGO: Reduced graphene oxide; ERGO: Electrochemically reduced graphene oxide; SiO_2: Silica nanoparticles; GA: Glutaraldehyde; Thi: Thionine; MIP: Molecularly imprinted polymers; AAM: Acrylamide; MBA: *N,N′*-methylenebisacrylamide; APS: Ammonium persulphate; PPy: Polypyrrole; MB: Methylene blue; GEM91: 25-mer gene expression of modulator 91; PTCA: 3,4,9,10-perylene tetracarboxylic acid; pDNA: Probe DNA; Ph-NH_2: 4-aminophenyl; CA: Carminic acid; AuNC: Gold nanoclusters; PV@CdS: PowerVisionTM polymer-encapsulated CdS QD; *o*-PD: *o*-phenylenediamine; EuNC: Europium sulfide nanocrystals; PAA: Poly acrylic acid; PABA: *p*-aminopenzoic acid.

3.3.2.2. Fluorescent biosensors. The field of fluorescent biosensors has been a growing research area over the last three decades. A wide range of books and articles has been published by experts in this field who have highlighted the advantages of fluorescent sensing over other optical methods. Fluorescence is by far the method most often applied to the biosensing and comes in a variety of schemes. Nowadays, one of the most common approaches in the field of optical biosensors is to combine the high sensitivity of fluorescence detection in combination with the high selectivity provided by bioreceptors. Thus, the fluorescent biosensors have less response time with high selectivity and sensitivity.

In this section, we deal with reviewing our recent results on the implementation of such biosensors with emphasis on the ones that use nanomaterials as fluorophore and quencher and can be used for detection of HIV infection. Table 3 presents the response charateristics of a list of nanomaterials-based biosensors for fluorescent detection of HIV biomarkers (87–110).

3.3.2.3. Electromagnetic piezoelectric biosensors. Over the last two decades, considerable progress has been made in the development of novel acoustic sensors, including piezoelectric based sensors. However, the detection capability of these sensors is fundamentally limited by their minimal detectable pressure. Therefore, there is still a great need to develop efficient sensing platforms and functional materials that can overcome the limitations of current acoustic sensing technologies. Self-assembling monolayer (SAM) chemistry is regularly regarded as a method of choice for the fabrication of these biosensors. In fact, such chemistry offers one of the highest quality routes for the preparation of chemically stable and structurally well-defined functionalizable organic surfaces, onto which the biomolecules such as proteins, antibodies or oligonucleotides can be attached in a subsequent immobilization step. Sheikh et al. [111] described a label-free piezoelectric acoustic immunosensor for detection of HIV-2 using a HIV-2 epitope-functionalized mixed self-assembled monolayer-coated quartz wafer (Fig. 3). The obtained results clearly revealed that the prepared immunosensor is effective in detecting and distinguishing HIV-2 from

Table 3
A list of nanomaterials-based biosensors for fluorescent detection of HIV biomarkers.

HIV makers	Type of affinity assay	LOD	DLR	References
$CD4^+$ cells	ICS-based sensor	34 cells μL^{-1} 44 cells μL^{-1}	50–1000 cells μL^{-1} (in PBMC) 100–800 cells μL^{-1} (in whole blood)	[87]
HIV-1 Protease	Carboxylated GO/Peptide-FAM	1.18 ng mL^{-1}	5–300 ng mL^{-1}	[88]
HIV-1 reverse transcriptase	Aptamer beacons/TAMRA	–	0.5–5.0 μM	[89]
HIV-1 Tat protein	3D carbon micropillar array platform/Capture aptamer/ Tat/Fuorescence-labeled aptamer	50 pM	0.05–100 nM	[90]
P24	EuNPs/Ab based microtiter-plate	3.7 pg mL^{-1}	Up to 500 pg mL^{-1}	[91]
	Microwell plates or microfluidic chip (Ab_1/p24/ Biotinylated Ab_2/Streptavidin-CDs)	30 pg mL^{-1}	30–1000 pg mL^{-1}	[92]
	Ab_1/p24/Biotinylated Ab_2/Streptavidin-conjugated FSN	8.2 pg mL^{-1}	10–1000 pg mL^{-1}	[93]
	Ab1/p24/Biotinylated Ab_2/Streptavidin-conjugated AuNC	5.0 pg mL^{-1}	Up to1000 pg mL^{-1}	[94]
Gene	Cu-MOF(*N*,*N*-bis(2-hydroxyethyl) dithiooxamidato copper (II))/ROX-pDNA(Molecular beacons)	0.22 nM	1–10 nM	[95]
	Guanine-rich pDNA(Molecular beacons)-AgNC	4.4 nM	5–200 nM	[96]
	RGO–ZnAl-LDH nanocomposite/$Ru(phen)_3Cl_2$/pDNA	59 ng mL^{-1}	0.8–18.0 μg mL^{-1}	[97]
	FAM-Hairpin pDNA/T7exonuclease/GO	38.6 pM	50–2000 pM	[98]
	pDNA/AgNCs/Autonomous exonuclease III	35 pM	50 pM–5 nM	[99]
	$pDNA_1$-AgNCs/$pDNA_2$-AgNCs/CNPs oxide	0.4 nM	1–50 nM	[100]
	Zwitterionic 1D/2D polymer co-crystal/FAM-pDNA	1.42 nM	1–120 nM	[101]
	3-mercapropionic acid-coated CdTe QDs/CDs/MTX/HIV dsDNA	1.0 nM	Up to 50 nM	[102]
	CDs/pDNA/AuNPs or AuNPs-GO	15 fM	50.0 fM–1.0 nM	[103]
	$pDNA_1$-AgNCs/$pDNA_2$-AgNCs/GO	1.18 nM	1–100 nM	[104]
	DNA(Molecular beacons)-AgNCs	5 nM	5–100 nM	[105]
	DNA-AgNC	3.18 nM	15–150 nM	[106]
	Zeolitic imidazolate framework(ZIF-8(zinc-methylimidazolate framework-8))/FAM-pDNA	1.2 nM	10–100 nM	[107]
	CdTe QD-based LFA	0.76 pM	1 pM–10 nM	[108]
	G-rich DNA_1-AgNCs/G-rich DNA_2 AgNCs	11 pM	0.2–700 nM	[109]
	TMSDRs combined with a non enzymatic target recycling amplification strategy	1.9 pM	10 pM–1 μM	[110]

ICS: Immunochromatographic strips; PBMC: Peripheral blood mononuclear cells; FAM: Fluorescein amidite; TAMRA: 5-Carboxytetramethylrhodamine; Ab: Antibody; Eu NPs: Europium nanoparticles: 3D: Three-dimensional; CD: Carbon dots; FSN: Fluorescent silver nanoparticles; AuNC: Gold nanocluster; MOF: Metal–organic framework; QD: Quantum dot; ROX: Carboxy-X-rhodamine triethylammonium salt; pDNA: Probe DNA; cDNA: Complementary DNA; AgNC: Silver nanocluster; LDH: Layered double hydroxides; RGO: Reduced graphene oxide; CNPs: Carbon nanoparticles; CdTe QDs: Cadmium telluride quantum dots; MTX: Mitoxantrone; LFA: Lateral Flow Assay; G: Guanine; TMSDRs: Toehold-mediated strand displacement reactions.

HIV-1 monoclonal antibodies with good selectivity in a real-world detection (serum) scenario. In a similar experiment, a quartz crystal microbalance (QCM) technique was employed for the determination of HIV-1 antigen at a very low concentration using AuNPs as a signal enhancer [112]. Here, the HIV-1 antigen was captured by a polyclonal antibody on the QCM surface that had been previously treated with 11-mercaptoundecanoicacid (MUA) and streptavidin. By using streptavidin-Au as an amplifier, the detection limit reached 1 ng mL^{-1}. The resulting calibration curve showed a reasonable linearity (1–10^7 ng mL^{-1}) with a determinant coefficient of 0.991.

3.3.2.4. SERS biosensors. In the past two decades, with providing the capability of enormous chemical structural information and a high sensitivity at single-molecule level, the surface-enhanced Raman

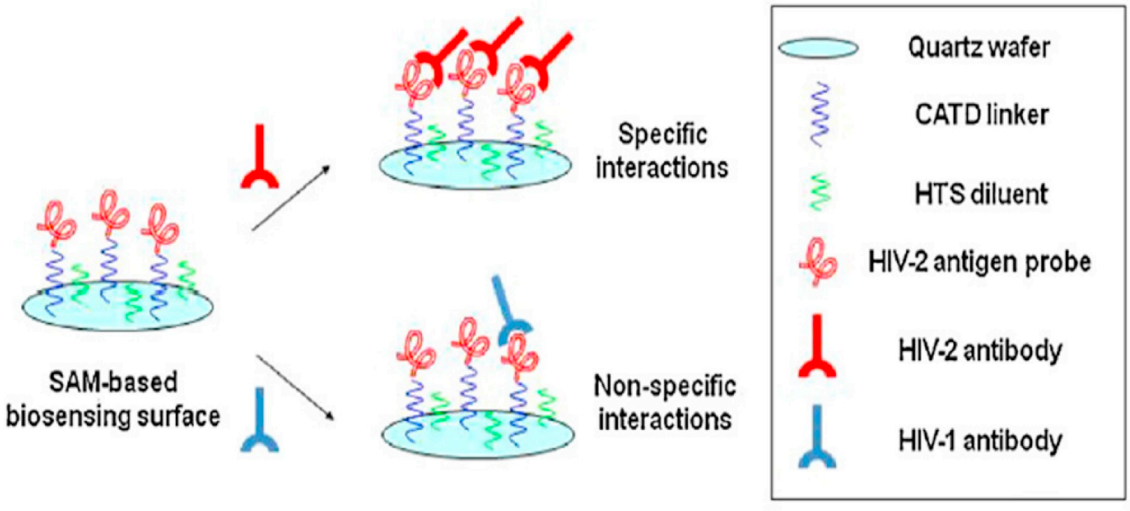

Fig. 3. Schematic representation of the HIV-2 epitope-functionalized mixed SAM-based electromagnetic piezoelectric acoustic biosensing platform; Reprinted by permission of Publisher.

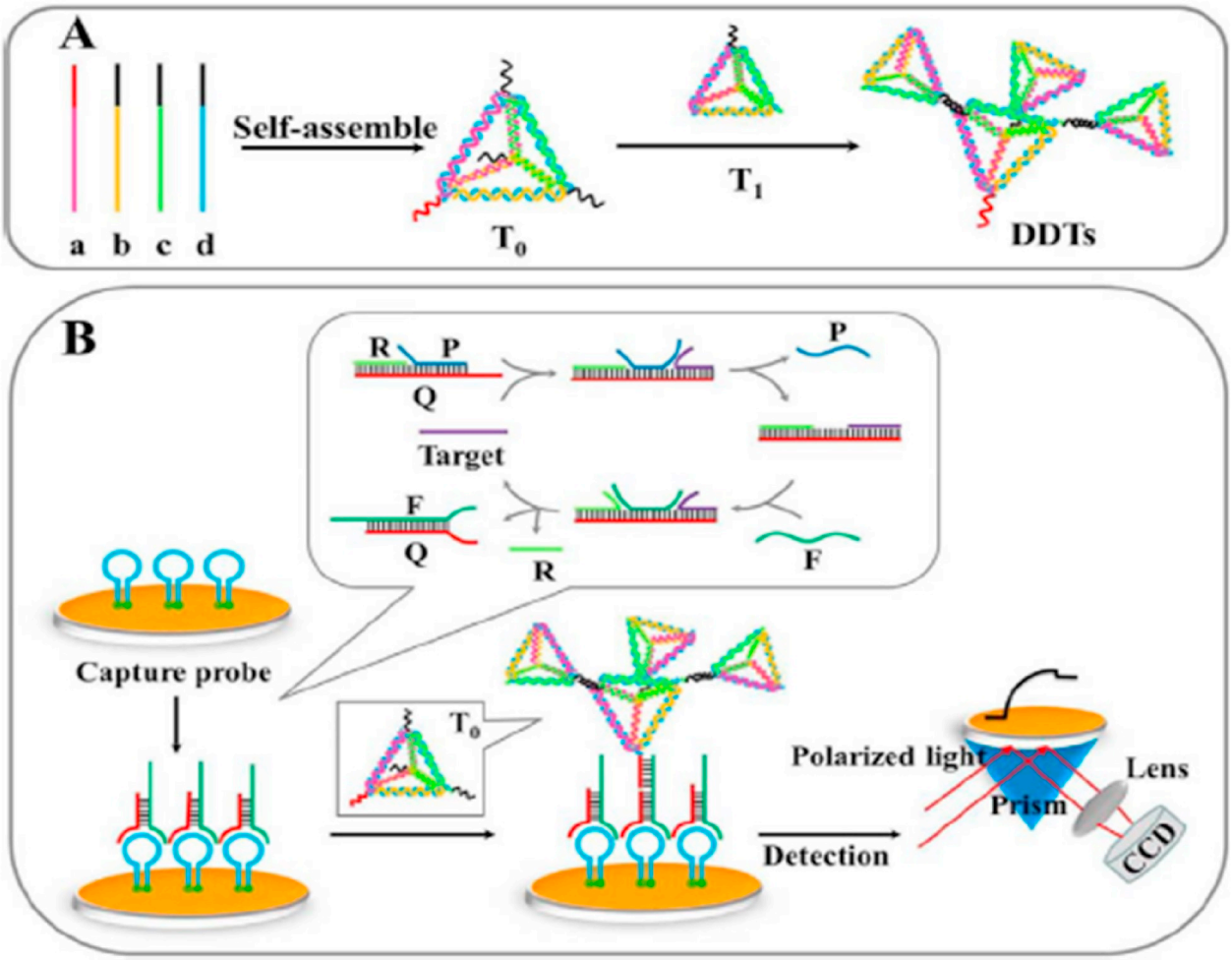

Fig. 4. Schematic illustration of the preparation of DDTs nanostructure and (B) Schematic representation of SPR biosensing strategy for HIV-related DNA detection based on ESDRs and DDTs nanostructure; Reprinted by permission of Publisher.

spectroscopy has developed into a significant tool in biomedical analysis, from small bioactive molecules to DNA, proteins and cells. The extrinsic SERS detection can provide further advantages over conventional fluorescence-based assays, including (1) Raman peaks typically have 10–100 times narrower spectral widths than the fluorescence labels, thus minimizing the overlap between different labels and increasing multiplex capability, (2) when the laser excitation wavelength is matched with the substrate LSPR wavelength, strong SERS signal is achieved from any SERS-active molecule within the zone of electromagnetic enhancement, thus a single source can be used for multiple labels, and (3) SERS labels are not susceptible to photobleaching [113]. Despite the fact that SERS has the capacity for biosensor signal transduction, there are some real limitations to the widespread adoption of SERS biosensors. Based on the fundamental cross-sections of fluorescence and Raman scattering, even a small amount of fluorescence has the potential to mask SERS signals. Clearly, this problem can be ameliorated by using infrared excitation or metallic NPs/surfaces to quench fluorophores or by performing confocal SERS, where a smaller sample thickness is probed. However, if the fluorophore is not in direct contact with the SERS substrate, background fluorescence will result. In addition, the practical issue of instrumentation cost limits the use of SERS. While there is precedent for inexpensive Raman spectrometers, for the most part, the cost of a laser, optics, spectrograph, and detector is high and, thus, it is prohibitive to many researchers [113].

One of the key points for the SERS application in these biosystems is how to prepare stable Raman labeled substrate with high SERS activity. Normally, molecules with large Raman cross sections and Ag- and Au NPs with high SERS activities have been applied as labels and substrates, respectively [114–116]. Traditionally, Raman labels and capture probes are either coadsorbed on the NPs surfaces or conjugated and then adsorbed on the NPs [117].

On the basis of SERS enhancement of Au NPs, Hu et al. [118] developed a sensitive nanojunction based method for determination of HIV gene related DNA. This subattomolar biosensing assay was based on multilayer metal-molecule-metal nanojunctions. Two complementary probes acted as bricks to build up the multi-metal-molecule-metal nanojunctions between AuNPs. They created SERS “hot spots” by the conjugated AuNPs, and also decreased the distance between AuNPs and Raman labels. Therefore, the Raman signal of the tag molecules on these detection probes was significantly enhanced due to the distance dependent electromagnetic enhancement of SERS. With regards to a HIV-1 DNA sequence, the platform could detect a concentration as low as 10^{-19} M (much lower than 1 fM) with the ability of single base mismatch discrimination. The SERS signal increased almost linearly from 0 to 10^{-13} M, and then reached a saturation stage at higher concentrations. Raman reporter-labeled AuNPs were also employed as SERS nanotags for targeting the HIV-1 DNA marker by Fu et al. [119]. They developed a novel SERS-based lateral flow assay for the quantitative analysis of this biomarker by monitoring the characteristic Raman peak intensity of the DNA-conjugated AuNPs. Under optimal conditions, a linear relationship between the Raman intensity and the HIV-1 DNA concentration was obtained over the range of 8 pg mL^{-1} to 64 ng mL^{-1} with a detection limit of 0.24 pg mL^{-1}.

Silver-containing metal nanostructures with strong and stable SERS signals hold great promise for developing ultrasensitive probes for biodetection. Su and co-workers [120] developed multicolor DNA-mediated Au–Ag nano-mushrooms that can act as ready-to-use SERS nanoprobes for ultrasensitive and multiplex DNA/RNA detection. They demonstrated that these SERS nanoprobes have specific hybridization properties because of the inherent ability of DNA/RNA hybridization with exposed oligonucleotides on AuNPs. In fact, the DNA involved in the nanostructures can not only act as a mediated DNA, but also act as a probe DNA and allow it to have an inherent ability to recognize DNA targets. Since many Raman-reporter molecules cannot be labeled to oligonucleotides, this study was the first establishing an efficient protocol in which thiol containing Raman reporters were co-assembled with the probe DNA. In addition, it showed the successful simultaneous detection of the three virus DNA targets in one sample.

Raman spectroscopy can be applied in rapid screening of blood for HIV before transfusion or for the blood bank. For example, Otange et al. [121] investigated the application of conductive silver paste smeared glass slides as Raman spectroscopy sample substrates for label-free detection of HIV-1 p24 antigen in blood plasma. The characteristic Raman spectrum of HIV-1 p24 antigen displayed prominent bands that were assigned to RNA and proteins that constitute the antigen.

3.3.2.5. SPR biosensors. Surface plasmon resonance is an optical non-destructive method that can detect very small changes in the refractive index. SPR has become an important biosensing technology due to its real-time, label-free, and sensitive nature. The light-based SPR biosensors can be used for the early detection of malignant and viral diseases and health care monitoring. Diao et al. [122] reported an innovative SPR biosensing strategy for highly sensitive detection of HIV-related DNA based on entropy-driven strand displacement reactions (ESDRs) and double-layer DNA tetrahedrons (DDTs). As it is seen in Fig. 4, ESDRs as enzyme-free and label-free signal amplification circuit were specifically triggered by the target DNA, leading to the cyclic utilization of target DNA and the formation of plentiful dsDNA products. Subsequently, the dsDNA products bind to the immobilized hairpin capture probes and further combine with DDTs nanostructures. Due to the high efficiency of ESDRs and large molecular weight of DDTs, the SPR response signal was enhanced dramatically. The proposed SPR biosensor could detect target DNA sensitively and specifically in a linear range from 1 pM to 150 nM with a LOD of 48 fM. In addition, the whole detecting process found to be accomplished in 60 min with high accuracy and duplicability. In particular, the developed SPR biosensor was successfully used to analyze target DNA in the complex biological sample, indicating that the developed strategy is promising for rapid and early clinical diagnosis of HIV infection.

3.3.2.6. Visual biosensors. Visual detections have recently attracted a great research attention, due to their convenient monitoring of the target analytes without using any advanced instruments. These assays are fast, inexpensive and portable. However, achieving visual detection of trace amounts of DNA targets with PCR-like sensitivity has remained as a major challenge. The first example of visual DNA detection scheme was demonstrated by Mirkin and co-workers by using DNA-functionalized AuNP probes [123]. The AuNP-based visual DNA detection methods still encounter the limitations of false positive signal outputs due to nonspecific aggregation of the functionalized AuNPs in complex biological media, time-consuming preparation (24–48 h) and stability of DNA-functionalized AuNP probes [124]. To explore solutions for the challenges encountered in current visual DNA detections, Zhou et al. [124] reported a quadratic signal amplification strategy for sensitive visual detection of HIV DNA biomarkers based on exonuclease III (Exo III)-assisted DNA recycling amplification and DNAzymes (Fig. 5). The presence of the target HIV DNA led to two independent and simultaneous DNA recycling processes to achieve

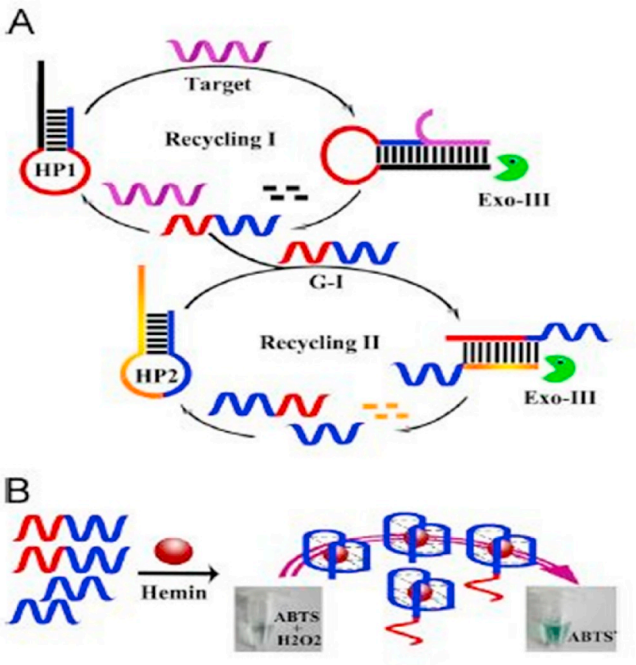

Fig. 5. Principle of the quadratic amplification strategy for sensitive and visual detection of HIV DNA. (A) The target HIV DNA-triggered, ExoIII-assisted quadratic amplification generation of massive G-quadruplex sequences and (B) The formation of numerous DNAzymes upon the addition of hemin and subsequent catalytic conversion of colorless $ABTS^{2-}$ to significantly intensified green color $ABTS^{\bullet-}$ for visual detection of trace amounts of HIV DNA; Reprinted by permission of Publisher. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

quadratic signal amplification with the assistance of Exo III. This quadratic signal amplification resulted in catalytic cleavage of the G-quadruplex sequence-locked hairpin probes to release numerous active G-quadruplex sequences, which further associate with hemin to form DNAzymes and cause significantly intensified color change for sensitive and visual detection of HIV DNA down to 2.5 pM. The proposed visual detection method employed unmodified hairpin DNA as probe, avoided using any complex and expensive instruments for signal transduction and was essentially simple. This method also showed a single-base mismatch discrimination capability as well.

3.4. Point-of-care detection of HIV

The fast and simple monitoring for infection has now become one of the key challenges for the diagnosis and treatment of infectious diseases. However, the debate has recently mounted regarding the nature as well as the value of laboratory monitoring in developing countries. Furthermore, the concept strategies in laboratory monitoring could be completely revolutionized in the near future by the emergence of simple, accurate and affordable monitoring tools, such as the rapid POC assays currently in the pipeline.

POC tests offer potentially substantial benefits for the management of infectious diseases, mainly due to shortening the time to result and making the test available at the bedside or at remote care centers. It can also be done without the need for trained personnel and advanced infrastructure [125]. Commercial POC tests are already widely available for the diagnosis of bacterial and viral infections and for parasitic diseases, including malaria [126]. Further evolution of POC tests may lead to new diagnostic approaches, including mobile phone sensing methods.

The mobile phone as detection element of POC device has been a burgeoning trend recently [127]. As an almost indispensable

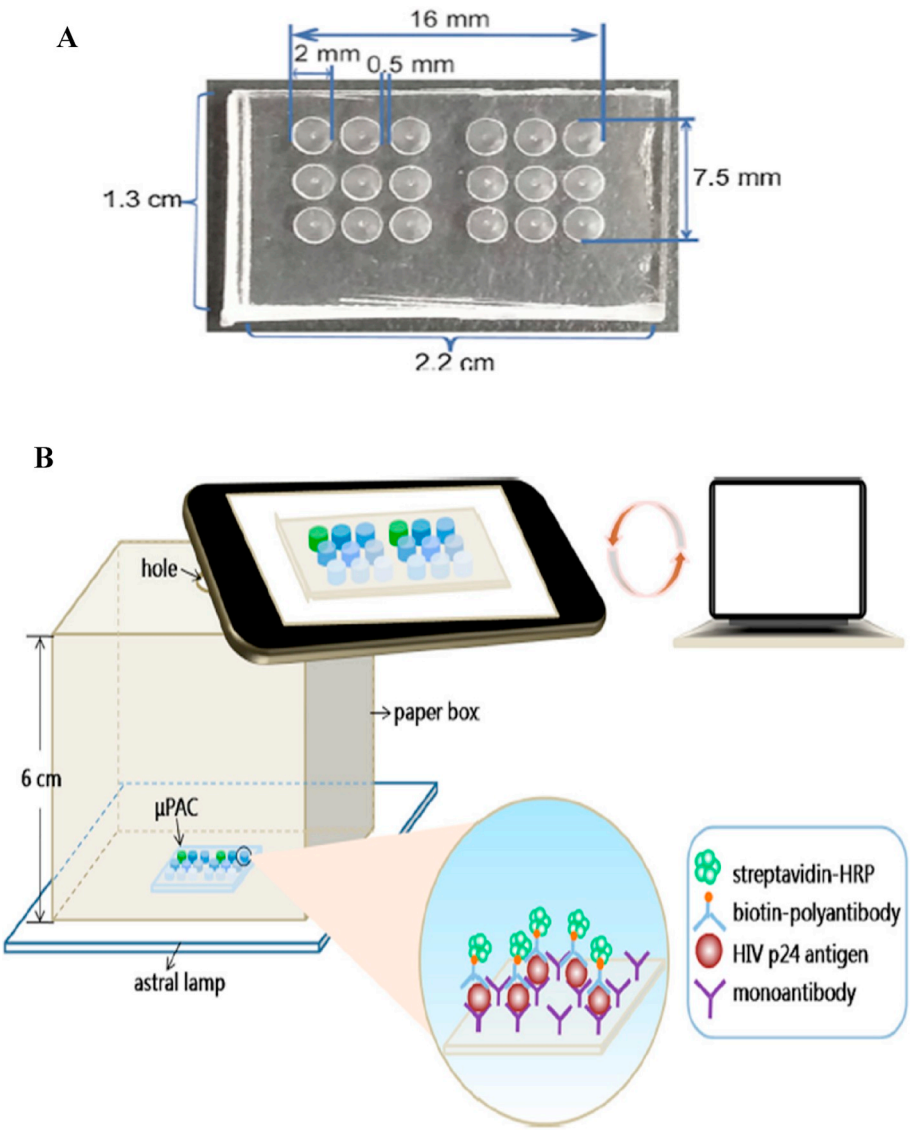

Fig. 6. (A) Photograph of the μPAC; (B) Schematic illustration of POC Sensing based on μPAC device mediated by a mobile phone; Reprinted by permission of Publisher.

communication tool, mobile phones are extremely popular and possess advantageous features of small size, intelligent function and low cost. More importantly, their capability of exchanging information through telecommunication network worldwide and access the internet easily make the data delivering over long-distance effectively. All these features are essential for a real POC device. Therefore, incorporation of a mobile phone into a detection platform can provide an alternative way of low-cost long-range diagnosis and may eliminate its dependence on the professional instruments. Efforts have been devoted to the fabrication of mobile phone-based POC devices [128–130].

A low-cost, portable, miniaturized, intelligent and sensitive POC detection apparatus is necessary for early diagnosis of HIV that is responsible for human AIDS. In this regard, Li and co-workers [131] presented a simple, low cost and portable immuno-testing platform employing an integrated device based on a mobile phone and micropit array chip (μPAC). The μPACs with a size comparable to common test strips were fabricated on transparent cyclic olefin copolymer sheets to ensure the optical detection and solvent compatibility (Fig. 6A). An antibody (for p24 antigen) was covalently immobilized onto the inner surface of micro-pits for sandwich immunoassay. The enzyme-catalyzed reaction was imaged by a mobile phone and the captured pictures were sent to a computer (server) for quantitative analysis (Fig. 6B). Based on this device, a colorimetric immunoassay was achieved for p24 antigen with detection limits of 190 and 650 pg mL^{-1} in the buffer and spiked human serum, respectively. The calibration curve was linear over the range of 128 pg mL^{-1}–400 ng mL^{-1}. Low-cost of μPACs and easy operation combined with wide coverage of mobile phones network all over the world make the proposed immunosensor a competent candidate as a diagnostic tool in resource-limited settings.

The advantages of the proposed method include its convenience, portability, and isolation of on-site detection and professional data analysis.

4. Conclusion

Rapid, sensitive and specific diagnostic assays play an indispensable role in the determination of HIV infection stages and evaluation of efficacy of antiretroviral therapy. To achieve these goals, many efforts have given to develop biosensors based on the specific interaction between bioreceptors and analytes. Several works have been performed to detect infection based on DNA hybridization or HIV-related proteins assay; however, fewer reports on the detection of virus particles are available in the literature. In fact, a large number of indirect methods have been developed for early diagnosis of HIV. These methods include

the determination of HIV type 1 or HIV type 2 antibodies, viral DNA (RNA), viral P24, p17, HIV-related enzymes and $CD4^+$ T lymphocytes counting. Interest in nanotechnology has revealed new possibilities in developing these biosensing assays. The higher surface-to-volume ratio, electrical and optical properties of nanostructures increases the sensitivity of biosensors. Carbon nanostructures, quantum dots, nanoclusters, metallic and metal oxide nanoparticles are merely some of the familiar materials that are emerging as candidates to develop highly sensitive HIV biosensors. The intelligent use of such nano-objects led to clearly enhanced performances with increased sensitivities and lowered detection limits of several orders of magnitudes. Despite significant progress in HIV biosensing in the last years, there is a great need for the development of POC technologies that are affordable, robust, easy to use, portable, and of sufficient quantitative accuracy to enable clinical decision making. In addition, the development of biosensors that allow for simultaneous detection of multiple HIV markers can achieve higher detection sensitivity while reducing false positives.

Acknowledgements

We greatly appreciate the supports of this work by Research Councils of Nuclear Science and Technology Research Institute (Tehran, Iran) and Razi University (Kermanshah, Iran).

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