REVIEW

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Functionalized gold nanoparticles: promising and efficient diagnostic and therapeutic tools for HIV/AIDS

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Functionalized gold nanoparticles are recognized as promising vehicles in the diagnosis and treatment of human immunodeficiency virus (HIV) owing to their excellent biocompatibility with biomolecules (like DNA or RNA), their potential for multivalency and their unique optical and structural properties. In this context, this review article focuses on the diverse detection abilities and delivery and uptake methodologies of HIV by targeting genes and proteins using gold nanoparticles on the basis of different shapes and sizes in order to promote its effective expression. In addition, recent trends in gold nanoparticle mediated HIV detection, delivery and uptake and treatment are highlighted considering their cytotoxic effects on healthy human cells.

1. Introduction

Human immunodeficiency virus (HIV) is a lentivirus belonging to a family of retroviruses which causes acquired immunodeficiency syndrome (AIDS) in humans. It is considered pandemic by the World Health Organization (WHO).¹ Recently, the dramatic rise in the number of HIV infections has been reported world-wide especially in the Middle East, North Africa, Eastern Europe, and Central Asia and is deeply perilous to human society.² The predominant infection is created *via* almost all the vital cells of the human immune system like helper T cells (specifically CD4⁺ T cells), macrophages, and dendritic cells. This is executed predominantly through the following three mechanisms:

1. Direct killing of infected cells.
2. Rise in apoptosis of infected cells.
3. Killing of infected CD4⁺ T cells *via* CD8 cytotoxic lymphocytes that recognize infected cells.

As the CD4⁺ T cell levels lessen beyond a certain edge, the cell moderated immunity is disoriented and the human immune system becomes susceptible to possible infections.³

The increasing number of new infections is generally due to a lack of awareness concerning the HIV infection, which apparently does not exhibit symptoms but is highly infective. The development of HIV infection takes place in three stages: (i) acute HIV infection, with flu-like symptoms, muscle pains, rash, and is observed for 3–6 weeks succeeding the infection, (ii) clinical dormancy, which manifests mild or no symptoms, and lastly (iii) AIDS, which is characterized by CD4 cell counts dwindling below 200 cells per mm³ of blood and/or

one or more major illnesses.^{4,5} The unavailability of HIV diagnostic assays generates complications, which can be used at the point-of-care (POC). However, initiating ART treatment in the early phase of the viral infection can prompt HIV diagnosis, thus ensuring high survival rates in the infected population and significantly reducing the spread of the virus. ART has demonstrated over 96% efficacy in reducing HIV spread in infected individuals.^{6,7}

Two closely concomitant kinds of HIV, namely HIV-1 and HIV-2, have been recognized. HIV is by far the most common cause of AIDS, but HIV-2 varies in genomic structure and antigenicity.⁸ The nucleocapsid or the viral core is enclosed by a viral envelope of this virus. The external layer of this viral core is composed of a protein p17, while the inmost core is composed of p24 proteins. The core of the viral particle confines the HIV genome, which comprises two single-stranded RNA moieties. The proteins involved here are referred to as antigens (*e.g.*, the p24 Antigen) as they can promote an immune response from the infected host.⁹

Prevention of infection is a crucial aspect for controlling AIDS. Therefore, a sensitive and practical detection method to monitor, diagnose, and screen HIV infection is especially important for controlling AIDS. Common methods for screening HIV infection in clinical practice include enzyme-linked immune sorbent assay (ELISA) based on a colour reaction and quantitative fluorescence polymerase chain reaction (PCR) based on nucleotide amplification. ELISA is an accurate and high-performance method, but has some drawbacks such as tedious procedures, high-volume sample consumption, a long time requirement, low sensitivity, and a long detection.³ Conjugating gold nanoparticles with this immunosensor can result in high sensitivity detection at low concentration, no complication and less time-consuming steps.¹⁰

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Classification of AIDS is carried out based on people exhibiting positive signals for two gp41, gp120/160 and p24, during anti-HIV antibody detection.^{9–12} Natural neutralizing antibodies were found in some HIV-infected individuals¹³ and one of these, called 2G12, was able to neutralize a broad range of HIV isolates. 2G12 recognizes an unusually dense cluster of carbohydrates on the ‘silent’ face of the viral gp120 envelope glycoprotein providing protection against viral challenge in animal models.^{14–16} Combined studies employing different techniques also revealed that 2G12 is capable of binding different arms of Man9GlcNAc2 with different binding modes, which would provide more opportunities to mimic the multivalent interactions between the antibody and the gp120 oligomannose clusters.¹⁷

In depth, the toxicity due to adverse drug interaction of combination therapy, poor oral bioavailability, multidrug resistance due to high genetic diversity of HIV-1, constant mutation and incompetent drug diffusion processes, inadequate residing time in the latent virion infected cellular areas, are the major disadvantages. Besides, the most significant drawback is that upon discontinuing treatment or drug resistance, even with highly effective antiretroviral therapy (HAART), the viral load rebounds into the blood.^{16–19}

Existing conventional approaches of HIV diagnosis have their restrictions such as: i) CD4 assays cannot diagnose virologic failure, ii) the RTPCR assays that are presently used to assess the viral load are time consuming and necessitate specialized facilities and qualified staff. However, nanotechnology can overcome these drawbacks by designing sensors and improving their sensitivity. Nanomaterials offer simple conjugation of bioreceptors on their surfaces to assist the targeted sensing. Essentially nanomaterials are helpful in the development of fast-response sensors since they have ease of mass transfer at very short distances which creates the rapid response of nanomaterial-based sensors.²⁰

Extremely detailed nanoscale engineering formulating nanodevices and nanostructures sets about the diagnosis, prevention, treatment of diseases like HIV and control of human biological systems. Several characteristics make gold nanoparticles (AuNPs) highly attractive for clinical use, such as their small size that facilitates entry into tissues and cells, their inert nature that ensures little host response to the molecules, and their potential for multivalency which allows the simultaneous conjugation of different molecules in the nanoparticle surface and simultaneous delivery. The introduction of antiretroviral therapy (ART) into clinical practice had led to a huge improvement in the life expectancy of HIV-infected people. Thus, despite all the benefits that ART confers, improvements in ART can be made. Therefore, the capacity of AuNPs to enter into different cell types, cross the blood–brain barrier (BBB) and exert antiviral activity upon conjugation with an antiretroviral was studied.¹⁵ A sensitive impedimetric DNA biosensor for the determination of the HIV-1 gene was formulated by employing electrochemically reduced graphene oxide (ERGO) as a sensing object.¹³

HIV p24 antigens appear at an earlier stage of HIV infection than antibodies, which is due to the vehement replication of the virus followed by acute infection causing highly infectious viraemia. Early detection of HIV p24 would be of significance in blood screening, neonatal HIV infection, therapeutic efficacy and disease progression. Nonetheless, post-acute HIV infection, certain antibodies prevailing in the body integrate with p24 antigens to form immune complexes, allowing the free antigen concentration to become too low to be detectable, this period is called “window phase”.³ A sensitive assay for HIV-1 p24 antigen detection by inductively coupled plasma mass spectrometry (ICPMS) had been developed using a biotin–streptavidin (BA) system and gold nanoparticle (AuNP) based immunoassay. Detection of the HIV-1 p24 antigen would cut down the diagnostic window between the time of human immunodeficiency virus (HIV) infection and laboratory diagnosis compared with detection of the anti-HIV antibody. Hence, detection of the HIV-1 p24 antigen in serum or plasma is one of the traditional methods for early diagnosis of HIV-1 infection (Fig. 1).¹⁸

Adaptive lateral flow (LF) strips are being widely used for point-of-care (POC) self-diagnostics, but they have some disadvantages in their detection sensitivity and quantitative analysis as they only identify the high cut-off value of a biomarker by considering colour changes that are detectable *via* the naked eye. To resolve these shortcomings associated with LF strips, a novel surface-enhanced Raman scattering (SERS)-based LF assay was developed for the quantitative analysis of a specific biomarker in the low concentration range. Herein, human immunodeficiency virus type 1 (HIV-1) DNA was chosen as the specific biomarker. Raman reporter-labeled gold nanoparticles (AuNPs) were used as SERS nano tags for targeting and detecting the HIV-1 DNA marker, in contrast to using bare AuNPs in LF strips.¹⁴

NPs are more resistant to nucleases compared with free aptamers (apts);^{21,22} hence another feature of Apt-NPs is their multivalent binding capability, resulting in increased biological activity compared with free Apts. Thus, each NP can be conjugated with different functional appts to prepare polyvalent ligand-protected NPs for multivalent binding.^{23,24} These structures express various unique properties which include cooperative binding, enhanced affinity, excellent catalytic properties for signal amplification, and extraordinary intracellular stability, which makes them convenient for intracellular molecular diagnostics and therapeutics.^{19,25,26} Implementation of split aptamers had been demonstrated coupled with gold nanoparticles (AuNPs) in this work for colorimetric detection of a transactivator of HIV (HIV-1 Tat).¹⁸ Various research studies on colorimetric assay for various target molecular detection by using AuNPs have been published recently.^{27–31} Specific recognition has been given to colorimetric assay with regard to the coordinated assembly and disassembly of aptamers on unmodified AuNPs for the detection of different targets as this method is label free, simple and time efficient.^{16,32–37} In continuation of our research work,³⁸ this review focuses on

Fig. 1 Functionalized gold nanoparticle mediated HIV detection and treatment.

advanced accomplishments in the field of treatment of a fatal disease called AIDS utilising gold nanoparticles that stand out as potential candidates. In addition, detailed mechanisms are discussed like HIV detection, p24 analysis, DNA biomarker detection, delivery and uptake followed by treatment *i.e.* both *in vitro* and *in vivo*.

2. Detection of HIV and its associated elements *via* AuNPs

2.1 Colorimetric detection by employing AuNPs

Before discussing functionalized AuNPs, it is fascinating to note that some studies have utilized unmodified gold nanoparticles for various detection studies, for example, to develop a fast and suitable method for detecting the activity of HIV-1 ribonuclease H (RNase H) colorimetrically.³⁹ Degradation of the RNA strand of the RNA–DNA hybrid is caused by the ribonuclease H (RNase H) activity of HIV-1 reverse transcriptase (RT) along with exhibiting a vital role in the virus replication cycle.⁴⁰ The basic mechanism can be explained as follows: if HIV-1 is inactive or absent, there will be no dissociation of the RNA–DNA duplex, resulting in accumulation of nanoparticles which is salt induced, along with a change in colour, whereas incubation of an artificial RNA–DNA duplex with HIV-1 RT results in cleavage of RNA (Fig. 2). At a fixed salt concentration, the corresponding fragments of single-stranded DNA (ssDNA) and single stranded RNA (ssRNA) on separation would develop a charged layer on the surface of nanoparticles thereby stabilizing them.

In this study, cleavage of RNA (in the RNA–DNA duplex) by RNase H was observed through polyacrylamide gel electrophoresis (PAGE). While studying how the activity of RNase H enzyme affects the stability of colloidal solutions, it was also found that deactivation of an enzyme can decrease stabilization of AuNPs (aided by an enzyme). Further, the absence of RNase H in the reaction mixture (containing AuNPs–14 nm) resulted in a colour change from red to blue, whereas in the presence of RNase H, accumulation of AuNPs was not observed.³⁹

RNA split aptamers along with AuNPs have also been employed for the colorimetric detection of gene regulatory

protein HIV-1 Tat. This protein exists in two different forms – Tat-2 exon (major) composed of 86 amino acids and Tat-1 exon composed of 72 amino acids (minor). This protein is found in the nucleus. Inside HIV, prevention of 5' LTR polyadenylation and activation of the LTR promoter happen as a result of interaction between transactivation response element (TAR) RNA and HIV-1 Tat.⁴¹ Moreover, for detecting or inhibiting HIV-1 Tat activity, aptamers are found to be suitable agents.⁴² The complex of aptamer and Tat is found to be more stable than that of Tat–TAR, with a 42-fold lower value of dissociation for the former.⁴³ Aptamers can also differentiate between molecules that are very similar to each other.⁴⁴ In this context, HIV-1 Tat has been detected in a dose-dependent manner, through a colorimetric assay based on unmodified AuNPs (10 nm) demonstrating a red to purple change in colour. In addition to the colour change, spectral changes were also analyzed with the help of a UV-visible spectrophotometer.⁴⁵ Aptamers adsorbed on AuNPs were able to prevent accumulation in the salt solution of NaCl,

Fig. 2 Unmodified AuNPs for the colorimetric detection of HIV-1 RNase H activity.³⁹

resulting in no change in the colour of AuNPs. Further, tests were conducted to estimate the specificity of split aptamers towards HIV-1 Tat by using Nef and p24 HIV-1 proteins for a comparative study, as detecting HIV-1 Tat can be hindered by these two proteins (Fig. 3). In the case of Nef, binding was not observed at all whereas with p24, a very small change in colour was observed during detection. On the other hand, a colour transition to purple was observed in the presence of Tat protein in solution along with an increased shift in spectral wavelength compared with those of Nef and p24, the reason for which was NaCl induced AuNP accumulation. Moreover, for analyzing the sensitivity of the method towards HIV-1 Tat, it was found that in the absence of HIV-1 Tat, the duplex generated by the addition of both aptamer strands separately was unstable. Apart from these studies, AuNPs have majorly been used in functionalized form through conjugation with suitable agents to achieve desired results in an efficient manner as described below.

2.2 p24 analysis for enhanced HIV detection

The viral envelope encompasses the nucleocapsid or the viral core which holds the HIV genome, made up of two single-stranded RNA molecules. The outer layer of this core consists of a protein called p17, while the inner core comprises p24 proteins. These are referred to as antigens (Ag) (*e.g.*, the p24 Ag) because an immune response is promoted from the infected host.⁴⁶ In this paper, an immunosensor has been developed using AuNPs, multi-walled carbon nanotubes (MWCNTs), and an acetone-extracted propolis film (AEP) for the detection of the p24 antigen (p24Ag) from HIV-1. The

AuNP/CNT/AEP composite film possessed good reproducibility, biocompatibility, activity and selectivity. Also, the detection of p24 in artificially positive human serum samples was done using the AuNP/CNT/AEP composite and was compared using electrochemiluminescence (ECL) technology which hence turned out to be a successful detection method.⁴⁷

The ECL method has been used for various purposes because of its high sensitivity, simple set-up and absence of a background optical signal. Several nanomaterials have been used to improve the performance of the analytical ECL method. For this purpose, SiO₂ nanoparticles (NPs) have been in the limelight owing to its properties such as a special pore structure, high surface area and excellent biocompatibility.⁴⁸ Furthermore, high amounts of Ru(bpy)₃²⁺ molecules were present in Ru-SiO₂ nanoparticles which had high sensitivity because they could greatly enhance the ECL signal. Limin Zhou *et al.* prepared the P-RGO@Au@Ru-SiO₂ composite, by combining gold nanoparticle-decorated graphene (PRGO@Au) and Ru(bpy)₃²⁺-doped silica (Ru-SiO₂) nanoparticles. This was a type of novel sandwich-type electrochemiluminescence immunosensor used for detecting the HIV-1 p24 antigen. A low ECL signal was observed, when Ru-SiO₂ nanoparticles were used. The ECL signal intensity increased when the P-RGO@Ru-SiO₂ composite was used. Additionally, the ECL signal enhanced when the P-RGO@Au@Ru-SiO₂ composite was used. Also, the P-RGO@Au@Ru-SiO₂ composite was used to analyze p24 in human serum, and the recoveries obtained were good in number.⁴⁹

Currently, common methods for the detection of p24 proteins are mostly based on enzyme-linked immune sorbent assay (ELISA) which is based on a colour reaction and

Fig. 3 Application of the split aptamer for the analysis of HIV-1 proteins. HIV-1 Tat, p24, and Nef were analysed.⁴⁵

quantitative fluorescence polymerase chain reaction (PCR). However, it has drawbacks such as long procedures, high-volume sample consumption, and low sensitivity.⁵⁰ In this paper, sandwich amperometric immunosensors have been developed based on the ELISA principle. In this detection scheme, the antigen is recognized by the primary antibody and enzyme (such as horseradish peroxidase [HRP])-labeled signal antibody to produce an immune complex which is "sandwich"-type. Using chronoamperometry, an electrode surface was electroplated with gold nanoparticles. Now, the trapped anti-p24 monoclonal antibodies were adsorbed on an electrode surface and bovine serum albumin (BSA) was used to block the remaining unconjugated active sites. HRP-labeled signal antibodies (HRP-anti-p24) were made to react with the electrode forming a sandwich immune complex (anti-p24/p24/HRP-anti-p24) on its surface.⁵¹

Overcoming some drawbacks of ELISA, the quartz crystal microbalance (QCM) is known for detection in the gas phase and aqueous solution proving to be an ultra-sensitive mass-measuring device.⁵² Conjugating gold nanoparticles with this immunosensor can result in high sensitivity detection at low concentration, no complication, and less time-consuming steps.¹⁰ In the current study done by Tan Nhiem Ly *et al.*, the QCM was used for the analysis in conjugation with AuNPs as a signal amplifier for HIV-1 p24 antigen detection. Streptavidin was conjugated onto AuNPs *via* covalent binding. Treatment of the QCM surface was done with 11-mercaptoundecanoic acid (MUA), thereafter immobilization was done using streptavidin, and subsequently a biotinylated polyclonal antibody was conjugated to capture the HIV-1 antigen. Hence, by using streptavidin-Au attachment, the signal was enhanced. Among other Au sizes (21, 30, 63, and 126 nm), the highest signal was yielded by streptavidin-Au with a size of 30 nm.⁵³

Also, an assay has been prepared using a AuNP based immunoassay through inductively coupled plasma mass spectrometry (ICP-MS) and a biotin-streptavidin (BA) combined system for the detection of the HIV-1 p24 antigen in human serum. The sensitivity of immunoassays is possibly enhanced with the use of the BA system, which relies on the elevated affinity of streptavidin for biotin, thereby improving enzyme-linked immunosorbent assay (ELISA).⁵⁴ Moreover, employing nanoparticle tags for the ICP-MS linked immunoassay results in enhanced sensitivity compared with metal ions, as each nanoparticle tag could contain a high number of atoms that can be detected.⁵⁵ An indirect determination of the p24 antigen was achieved, by releasing and detecting trapped AuNPs through ICP-MS, following a sandwich-like reaction between anti-HIV-1 p24 monoclonal antibody, p24 antigen, biotinylated anti-p24 polyclonal antibody and AuNP (15 nm)-labeled streptavidin. The specificity of the BA-ICP-MS technique was tested for the detection of the p24 antigen in comparison to that of other four proteins, namely human AFP, human CEA, human HSA, and human IgG. It was found that only p24 was identified in the immunoreaction and the other four proteins did not interrupt its detection, hence highlighting that this method was highly

efficient. Both ICP-MS linked immunoassay and BA-ELISA were employed for the detection of p24 by using human serum samples, where the results were found in agreement.⁵⁶

2.3 Role of antibodies in efficient detection methods

Kwon *et al.* developed a one-step label-free immunoassay for the point of care diagnosis of AIDS.⁵⁷ Engineered human ferritin nanoparticles were employed for this purpose, along with the introduction of the HIV antigen (gp41) on their surface leading to the formation of 3-dimensional probes, capable of detecting anti-HIV antibodies efficiently. Assay signals were self-enhanced by the use of gold ions (Au^{3+}) which were adsorbed on the surface of the hexa-histidine peptide (H_6), multiple copies of which are found on the probes. The functioning of this immunoassay was compared with that of the lateral flow assay (LFA). Among various antigenic epitopes of HIV, the HIV envelope glycoproteins are very promising, and gp41 (segment of transmembrane protein gp160) serves as a very important target of recognition by anti-HIV-1 antibodies.⁵⁸ Three HIV proteins, gp41, p24 and gp120, were used as probes for the detection of anti-HIV anti-bodies in this experiment. Classification of AIDS is done on the basis of people exhibiting positive signals for two gp41, gp120/160 and p24, during anti-HIV antibody detection.^{8,9} The diameter of colloidal AuNPs used was about 25 nm. Two additional antigen probes, hFTH-p24- H_6 and N-ePGKgp120, were used to increase the signal output, which improved the sensitivity to 90%. Secondly, one step immunoassay was employed, using engineered protein particles hFTH-(gp41)₃- H_6 and H_6 -SPA_B-capsid contained in a solution. Au^{3+} ions could be adsorbed on the surface of both protein particles.⁵⁹ No blue colour was observed on testing healthy sera, whereas tests conducted on patient sera revealed the fast appearance of blue colour which darkened with time.

In a recent study, a carboxymethylcellulose (CMC) biopolymer has been used in combination with AuNPs as an environment-friendly *in situ* reducing agent and surface stabilizing ligand for a colloidal method in aqueous medium to develop nano-immunoconjugates.⁶⁰ The Gp41 glycoprotein receptor or HIV monoclonal antibodies were employed to functionalize AuNPs-CMC nanocolloids (AuNPs-CMC-gp41 or AuNPs-CMC_PolyArg-abHIV) for efficient LIA detection. For vaccine development, as well as HIV and retrovirus detection, gp41 has gained prominence as a potential target due to its involvement in host cell invasion process.⁶¹⁻⁶³ It is a subunit of the envelope protein complex of HIV-1. Spherical colloidal AuNPs were developed with the average size ranging from 12–20 nm. The dynamic light scattering (DLS) method was used as a probe in this assay for *in vitro* detection of the HIV monoclonal antibody. A noteworthy rise in the hydrodynamic diameter value was observed on mixing colloidal dispersions of AuNPs-CMC-gp41 (antigen) and AuNPs-CMC_PolyArg-abHIV (HIV-1 antibody) which was detected by DLS because of the aggregate formation between immunoconjugates. The immunoassay was found to be highly sensitive in the nanomolar range.

2.4 DNA biomarker detection

A paper-based user-friendly lateral flow (LF) strip, combining nanoparticles and chromatographic separation, is helpful because nanoparticles help in amplifying signals, and hence are used for the analysis with high sensitivity and selectivity.^{64–66} LF strips have attracted significant interest because of their simplicity, rapid analysis, low costs, long-term stability over a wide range of climates, and no requirement of skilled technicians for them to be used.^{66–68} However, LF strips have certain drawbacks such as low sensitivity and limits in quantitative analysis.⁶⁹ Surface-enhanced Raman scattering (SERS) spectroscopy can be used to overcome these challenges because of its high sensitivity.^{70–72} Moreover, AuNPs due to their high SERS enhancement effect are used as an optical enhancing agent in SERS.^{71,72} In a research study, Xiuli Fu *et al.* proposed the use of MGITC-AuNPs-DNA SERS (malachite green isothiocyanate (MGITC))-functionalized AuNPs as SERS tags. For analysing HIV-1 DNA, the DNA was bound to the surface of AuNPs. The presence of HIV-1 DNA follows a colour change to red on the test line. Factors responsible for an effective analysis were: running buffer solution, DNA-conjugated AuNP concentration, and concentration of DNA. Furthermore, the comparison between the SERS-based lateral flow assay and the commercially available assay kit was done and it was concluded that the SERS-based LF assay was useful for an early diagnosis of HIV-1 since it is sensitive in a lower concentration range too.⁷³

Cy3-DNA present inside the HEK293 cells exhibited enhanced fluorescence intensity for PDDAC-Au NR-DNA and PEI-Au NR-DNA complexes, whereas cetyltrimethylammonium bromide (CTAB)-Au NR-DNA did not show any increase in fluorescence intensity with time.⁷⁴

2.5 Anti-HIV drug sensing and detecting other associated molecules

Gold nanorods (AuNRs)-chitosan (CHIT) have been deposited over a pencil graphite electrode (PGE) to develop an anti-HIV drug sensor with improved selectivity and sensitivity towards deferiprone (DEF) using the EIS (electrochemical impedimetric) method. DEF causes apoptosis and thereby prevents HIV-1 from replicating, due to which it is widely used for HIV-1 treatment.⁷⁵ 0.005–1000 μM was the selected range to analyze the pattern between anti-HIV drug concentrations and electron transfer resistance.⁷⁶ Initially, a quick decrease in electron transfer resistance was observed, which was attributed to a conductive route for electron transfer and desired orientation exhibited by AuNRs (80 nm). The charge transfer resistance (R_{CT}) value was higher in the absence of the enzyme than in its presence, indicating improved response by the sensor. A linear output was obtained from 100 to 1000 μM because of depletion in electron transfer resistance with increasing concentration of DEF.⁷⁶ No significant interference was observed in the presence of cholesterol, uric acid, urea and glucose as serum interferents while analyzing their impact in measuring drug levels.

Lee *et al.* developed a gold nanodot based LSPR immunosensor for efficient detection of HIV-1 virus-like particles (VLPs).⁷⁷ The sensor was label-free along with enhanced sensitivity and selectivity. Here, an electrochemical deposition was used to create a sensing layer of 10–20 nm circular Au nanopattern on a glass substrate coated with indium tin oxide (ITO). HIV-1 neutralizing gp120 monoclonal antibody fragments were used to functionalize the Au nanopattern surface through gold-thiol interaction in an oriented manner. In this way, interactions on the sensing layer altered the refractive index, thereby causing changes in absorbance leading to the determination of HIV-1 VLPs (Fig. 4).

An increase in the LSPR signal was observed using the above sensor. The absorbance increased on adding HIV-1 VLP concentrated solution to the modified sensing layer of the sensor, and went back to its initial value after employing KCL solution to break antibody-antigen bonds. The sensor was further tested with variable HIV-1 VLP concentrations, which resulted in an increased intensity of absorption over the selected range. Compared with the previous LSPR based virus detection approach, the limit of detection in this study was found to be 10-fold higher.^{78,79}

An immunochromatographic (IC) reaction and proteolysis activity of HIV-protease have also been combined to develop an IC strip test in order to detect HIV-1 protease inhibitors (PIs).⁸⁰ The proteolytic activity exhibited by the viral protease (PR) enzyme through selective cleavage of Gag and Gag/Pol precursors leads to the production of infectious HIV-1 viruses. Infectivity decreases,⁸¹ and defective viral particles are produced when protease activity is absent. The lack of infectious viruses occurs due to inactivation of HIV-1 PR activity.⁸² HIV PIs in the plasma levels of healthy and HIV-infected patients undergoing antiretroviral treatment with and without lopinavir (LPV, a protease inhibitor) were semi-quantified using the developed strip. Colloidal gold nanoparticles (10–30 nm) were conjugated to anti-MA HB-8975 mAb (anti-MA HB-8975-CGC), absorbed by the conjugated pad of the strip. On evaluating the strip with clinical samples of HIV-infected patients, PI concentrations were efficiently detected.

Due to the high sensitivity, rapidity, simplicity and no sample preparation, the electrochemical method is the most convenient method (Table 1).^{83–87} Jagriti Narang and coworkers constructed an amperometric biosensor, where the enzyme was immobilized on the surface of the AuNP- CaCO_3 hybrid, for the detection of deferiprone. A high sensing signal was observed in the case of the AuNP- CaCO_3 /silica sol-gel-modified GCE in comparison to a low sensing signal as shown in Fig. 5. Moreover, it showed quick response, an accuracy of 99% and stability of the biosensor even after a month.⁸⁸

3. Delivery and uptake of AuNPs for targeting HIV

Antiretroviral therapy (ART) has led to a huge improvement in the lifespan and quality of life of HIV-infected patients

Fig. 4 Depiction of the quantitative detection of HIV-1 particles via immunoassay configuration with the LSPR technique.⁷⁷

Table 1 Various detection methods used to detect numerous targets

S. No	Target detected	Determination method	AuNP size	Molecule used	Ref.
1	HIV-1 p24 antigen	BA-ICP-MS	15 nm	Anti-HIV-1 p24 monoclonal antibody, biotinylated anti-p24 polyclonal antibody and Au NP-labeled streptavidin	41
2	HIV-1 RT	Colorimetric detection	14 nm	Single-stranded DNA (ssDNA) and single stranded RNA (ssRNA)	39
3	Deferiprone	EIS	80 nm	Chitosan	76
4	HIV-1 VLPs	LSPR immunosensing	10–20 nm	HIV-1 neutralizing gp120 monoclonal antibody fragments	77
5	Anti-HIV antibodies	Label free one step immunoassay	100–1000 nm	Hexa-histidine peptide	57
6	HIV-1 TAT	Colorimetric detection	10 nm	RNA split aptamers	45
7	HIV monoclonal antibody	LIA	12–20 nm	gp41 receptor	60
8	Lopinavir and ritonavir	Immunochromatographic strip	10–30 nm	Anti-MA HB-8975 mAb	80
9	p24Ag	Electrochemiluminescence (ECL) technology		AuNP/CNT/AEP composite	47
10	HIV-1 p24 antigen	Electrochemiluminescence (ECL) technology	55 ± 3 nm	Ru-SiO ₂ nanoparticles and P-RGO@Ru-SiO ₂ composite	87
11	p24 proteins	ELISA	300 nm	Anti-p24/p24/HRP-anti-p24 electroplated with AuNPs	51
12	HIV-1 p24 antigens	QCM	21, 30, 63, and 126 nm	Streptavidin-Au	53
13	HIV-1 DNA	SERS-based LF assay	30–40 nm	MGITC-AuNPs-DNA SERS	73
14	AuNRs	Pegfp	15 nm	PDDAC-Au NR-DNA, PEI-Au NR-DNA, CTAB-Au NR-DNA	74
15	Deferiprone	Electrochemical method	Less than 100 nm	HRP/AuNP-CaCO ₃ /GCE	87

after its the introduction in the 1990s.⁸⁹ However, ART has limitations like the development of drug resistance and drug toxicity. Also, these drugs fail to get into some tissues resulting in the formation of viral reservoirs. Despite ART, HIV could penetrate into the CNS and might cause neurocognitive disorders.⁹⁰ Additionally, it is observed that the CNS could act as an HIV reservoir.⁹¹ Carolina Garrido *et al.* prepared AuNPs and conjugated them with an antiretroviral so that it could penetrate different cell types and cross the blood-brain barrier. Inductively coupled plasma-mass spectrometry (ICP-MS) was used to observe the accumulation of AuNP-TAMRA (gold nanoparticle-tetramethylrhodamine) and AuNP-glucose across the BBB. Accumulation of gold in the brain did not differ much

between mice injected with AuNP-TAMRA and AuNP-glucose in content. However, accumulation in the spleen decreased when AuNP-glucose was injected. AuNPs were conjugated with Cy5 or TAMRA (AuNP-Cy5 or AuNP-TAMRA, respectively) and incubated with different cell types – PBMCs (peripheral blood mononuclear cells), macrophages, and HBMECs (human brain microendothelial cells) – for evaluating cellular uptake using confocal laser scanning microscopy. As the concentration of AuNP-Cy5 was increased, progressive cellular uptake was observed. Moreover, no toxicity was observed at different concentrations of AuNPs conjugated or not with raltegravir.⁹²

Immature dendritic cells (iDCs) are the early targets of HIV-1.^{93,94} iDCs are involved in the transfer of HIV-1 to CD4+

Fig. 5 Addition of HRP onto AuNP-CaCO₃ hybrid and detection of deferiprone using the electrochemical method.⁸⁸

lymphocytes.⁹⁵ DCs express C-type lectin receptors (CLRs) like intercellular adhesion molecule-3 (ICAM-3) grabbing nonintegrin (DC-SIGN; CD209) constituting a major receptor for capturing HIV-1.^{96,97} Furthermore, DC-SIGN identifies N-linked high-mannose glycan clusters on gp120 and has a single CRD.⁹⁸ To investigate the effect of the fluorescently labelled structure of the mannoAuNPs (FITC-mannoAuNPs) by targeting the DC-SIGN receptor on DCs, Blanca Arnáiz *et al.* conjugated FITC-mannoAuNPs with 50% density of linear di-(D), tri-(T), or tetra-(Te), or branched penta-(P) and hepta-(H) mannosides and detection was done using flow cytometry (FC) and confocal laser scanning microscopy (CLSM). No cytotoxicity was observed when incubation of iDCs, Raji, or Raji DC-SIGN cell lines was done with FITC-mannoAuNPs. Moreover, preferential uptake of FITC-Te50AuNP was observed (cellular fluorescence was monitored by FC) in comparison to the rest of the AuNPs.⁹⁹

Madhuri Sharon and team pegylated AuNPs with polyethylene glycol (PEG) as PEG proves to be beneficial than AuNPs alone since it prevents RES clearance, hides AuNPs from the immune system and enhances the biocompatibility of AuNPs. The MTT assay was done to check the cytotoxicity of AuNPs and pegylated AuNPs. It was observed that as the concentration of gold nanoparticles increases the cytotoxicity increases as well.¹⁰⁰

Ligeng Xu and team explored gold nanorods (AuNRs) as delivery vehicles in conjugation with cetyltrimethylammonium bromide (CTAB), poly(diallyldimethylammonium chloride)

(PDDAC), and polyethyleneimine (PEI). To monitor the transfection abilities of AuNRs, a plasmid-enhanced green fluorescent protein (pEGFP) was used. Due to the “proton sponge” effect, PEI-Au NRs and PEI showed higher transfection capability. A high transfection effect was also observed for PDDAC-Au NRs, but HEK293 cells could barely be transfected by CTAB-AuNRs. Intracellular trafficking of AuNRs was assessed using the red fluorescent probe Cy3-dCTP which was used to label pEGFP through the nick translation method, localization was observed using transmission electron microscopy (TEM) and cellular internalization was observed using inductively coupled plasma mass spectrometry (ICPMS). Studies have shown that PDDAC-Au NR-DNA and PEI-Au NR-DNA complexes showed better colocalization than the CTAB-AuNR-DNA complex. Moreover, the CTAB-AuNR-DNA complex was found in endosomes or lysosomes and some in mitochondria too, while PDDAC-AuNR-DNA and PEI-AuNRs-DNA complexes were present in lysosomes or endosomes. Additionally, the PDDAC-AuNR-DNA complex indicated having high internalization, which might be due to its small size.⁷⁴ Some of the conjugated molecules employed for delivery and uptake have been summarized in (Table 2).

3.1 HIV specific T cell response through AuNP induced DC

AuNPs have also been employed to transport HIV-1 peptides and high mannose type oligosaccharides (P1@HM) conjugated to AuNPs, through getting engulfed by monocyte-derived

dendritic cells (MDDCs) and generating an antigen specific T cell response¹⁰¹ (Fig. 6). This system tested the potential of DCs *ex vivo* to display processed HIV-1 peptides to T cells obtained from HIV+ patients. DCs were chosen for this study as they exhibit a vital role in initiating and regulating immune responses against harmful foreign microbes along with being efficient antigen presenting cells (APCs).¹⁰² Moreover, a very significant receptor for HIV-1 inside DCs is contained by a C-type lectin, known as dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN). Therefore, AuNPs were modified for this study, through conjugation with dimannose (Man α 1-2Man) and HIV Gag p17 protein, and had a mean diameter of 2.3 nm. Effects of this system on MDDCs obtained from HIV+ patients revealed that P1@HM was able to induce enhanced response by CD8+ T cells and even increased CD4+ T cell growth compared with the negative control, upon addition of maturation inducing cytokines, after P1@HM was engulfed by MDDCs. The release of pro-T_H1 (T helper 1) cytokines such as IL-2R, IL-12, IFN- γ and IL-15, rose by more than 3-fold due to the effect of P1@HM. In addition to this, the functionalized system also achieved improved secretion of T_H1 chemokines such as MIP-1 β (CCL4), CCL5 (RANTES), MIP-1 α (CCL3), MIG and IP-10 and pro-T_H2 (T helper 2) cytokines (by more than 2-fold) such as eotaxin, IL-13 and IL-5.

4. Treatment modalities for HIV using AuNPs

4.1 Gp120 interaction study with 2G12 mimic for HIV inhibition

An anti-HIV antibody, 2G12, capable of neutralizing a wide range of HIV isolates was utilized to study its interaction with HIV gp120 glycoprotein. 2G12 helps to identify dense clusters of carbohydrates on the 'silent' face of viral envelope glycoprotein gp120, thereby safeguarding against viral challenges in animal models.^{103–105} Many studies also depict that 2G12 can bind to different arms of Man₉GlcNAc₂ with various modes of binding, which creates more prospects to mimic the multivalent interactions between the gp120 oligomannose clusters and the antibody.^{106–108} Therefore, to reassemble Man₉GlcNAc₂ clusters of gp120 for enhanced viral envelope mimicking, its three arms, D1 (tetramannoside-Mana1–2Mana1–2Mana1–3Mana1–, Te), D2 and D3 arms (pentamannoside-Mana1–2Mana1–3[Mana1–2Mana1–6]Mana1–, P) were conjugated onto the surface of gold

nanoparticles (AuNPs) resulting in the formation of Te/P-AuNPs (glyconanoparticles). Binding between 2G12 and Te/P-AuNPs was analyzed using surface plasmon resonance (SPR) and saturation transfer difference NMR (STD-NMR) spectroscopy. The prepared Te/P-AuNPs contained a total of 10% oligomannoside with 5% of Te and 5% of P. With the help of transmission electron microscopy (TEM), the gold core was found to have an estimated average diameter of 1.8 nm. Controls were also prepared for a comparative study using AuNPs containing 10% Te, 100% 5-mercaptopentyl b-D-glucoside (Glc-AuNP), or 10% dimannoside Mana1–2Mana (D-10-AuNP). The interaction between Te/P-AuNPs and sensor chip fixed 2G12 was studied by SPR along with those of controls. At 10 $\mu\text{g mL}^{-1}$, Te-10-AuNPs generated 12 RU (response units) response, whereas a response of 35 RU was observed by Te/P-AuNPs at equal concentration. According to previous studies, no binding was observed between 2G12 and 10% P incorporating AuNPs at concentrations from 10–0.31 $\mu\text{g mL}^{-1}$.¹⁰⁹ A decrease in 2G12/gp120 interaction was evaluated using AuNPs. Inhibition of 2G12/gp120 interaction was observed on testing with Te-10-AuNPs (half maximal inhibitory concentration, IC₅₀ = 2.3 μM) and Te/P-AuNPs (8-fold depletion in IC₅₀ compared with that of Te-10-AuNPs) in a dose-dependent manner. On the other hand, P-10-AuNPs was unable to inhibit 2G12/gp120 interaction. Inhibition testing was performed at varying concentrations of P-10-AuNPs, Te/P-AuNPs, and Te-10-AuNPs (10 or 3 $\mu\text{g mL}^{-1}$). Therefore, Te/P-AuNPs was found to be the most suitable ligand due to the combinational effect of Te and P which was credited to the binding between 2G12 and the D3 arm of the pentasaccharide.¹¹⁰

4.2 The use of aptamers against HIV

Gold nanoparticles have also been combined with aptamers to combat HIV. Aptamers (short nucleic acid sequences) are fascinating drugs employed in gene therapy against HIV.^{111,112} Various nucleic acids interacting with HIV-1 reverse transcriptase (RT) have been analyzed by repetitive cycles of *in vitro* selection through systematic evolution of ligands by exponential enrichment (SELEX),^{113,114} out of which RT1t49 (Apt_{pol}) and ODN 93 (Apt_{RT}) were the two most promising single-stranded DNA (ssDNA) aptamers.¹¹⁵ In this context, Apt-NPs were synthesized by utilizing HIV-1 RT aptamers (Apt_{pol} or Apt_{RT}) and AuNPs (13 nm diameter). This conjugate system tested the polymerizing capability of HIV-1 RT. AuNPs were

Table 2 Delivery and cellular uptake characteristics by different AuNPs

S. No	Delivery and uptake	Determination method	AuNP size (nm)	Molecule used	Ref.
1	AuNPs-(Man α 1-2Man)-HIV Gag p17		2.3 nm	Dimannose (Man α 1-2Man) and HIV Gag p17 protein	101
2	Accumulation in spleen decreased when AuNP-glucose was injected	ICP-MS	1.8 $\pm$ 0.32 nm	AuNP-TAMRA and AuNP-glucose	92
3	Preferential uptake of FITC-Te50AuNP was observed	FC, CLSM	1.2–2.0 nm	FITC-mannoAuNPs	99
4	PEI-AuNRs and PEI showed higher transfection capability	pEGFP	15 nm	PEI-AuNRs, PDDAC-Au NRs, CTAB-Au NRs	74

Fig. 6 Generation of antigen specific T cell response and secretion of cytokines via P1@HM induced MDDCs.¹⁰¹

conjugated to aptamers containing 15 thymine linker length (T_{15}). Depletion in the activity of HIV-1 RT was observed till 25.3%, 32.9%, and 94.1% by employing 40Apt_{pol}- T_{15} -Au NPs, 40Apt_{RH}- T_{15} -AuNPs or 40rDNA- T_{15} -AuNPs (rDNA-random DNA), where AuNPs contained 40 aptamer molecules per nanoparticle. These inhibitory levels were higher compared with those of free aptamers. The efficiency of linkers was also analysed by comparing three different lengths (T_{15} , T_{30} , and T_{45}), out of which the highest potential for inhibition was shown by 40Apt_{pol}- T_{45} -AuNPs along with a minimal effect on activity upon exposure to DNase I. As the binding sites for two selected aptamers were different, both aptamers were tested in combinations to observe the inhibitory effects on HIV-1 RT. It was found that the highest inhibitory activity was exhibited by the combination of Apt_{pol} and 40Apt_{RH}- T_{45} -AuNPs. A lentivirus based on HIV was used to test the antiviral efficacy in HepG2 as host cells in which 96% approximate cell viability was observed on employing 40Apt- T_{45} -Au NPs (Apt_{pol} or Apt_{RH}). Even in comparison to an anti-HIV drug (abacavir), 40Apt_{pol}- T_{45} -AuNPs and 40Apt_{RH}- T_{45} -AuNPs showed improved inhibitory activity.¹¹⁶

AuNPs in conjugation with raltegravir and HIV inhibition were tested in PBMCs. It was observed that conjugating a larger number of RAL (modified raltegravir molecule, EL-04-109 (thiolated raltegravir)) molecules (33 or 66 RAL molecules) with AuNPs impaired the viral inhibition, in comparison to a small number of RAL molecules (4 RAL

molecules). A small number of RAL molecules (4 RAL molecules) inhibited the replication of HIV-1 down to 25.23%.⁹² Observations show that gel formulations of EGNz and man-EGNz were retained within the vaginal epithelium and inside vaginal mucosa, while some percentage was able to permeate through it. This microbicidal system proved to inhibit p24 as well as proved to be safe using a pre-clinical toxicology study.¹¹⁷ When AuNPs were used alone, the inhibition of the p24 antigen was observed more for post-treatment after interaction with the virus. On the other hand, pegylated AuNP effects on inhibition of p24 were more profound for pre-treatment.¹⁰⁰

4.3 In vitro targeting of HIV

HIV-1 virus's surface contains the receptor gp120 which is populated with glycoproteins. Meanwhile, lectin receptors are expressed on the surface of mononuclear phagocyte system (MPS) cells and hence are important hosts for HIV. The presence of sugar molecules on the receptor gp120 can be targeted by these lectin receptors.¹¹⁸ In this case, for the indirect targeting of the host cells protein (lectin)-carbohydrate (mannose) interaction has been used. A microbicide can be used for localization of the delivery system before the viral attack in the closest proximity of host cells.^{119,120} In the study done by T. Malik *et al.*, vaginal microbicides were prepared using efavirenz

Table 3 Inhibition and treatment by AuNPs in conjugation with various molecules

S. No	Characteristics	Target	AuNP size (nm)	Molecules used	Ref.
1	Inhibition of 2G12/gp120 interaction	2G12	1.8 nm	Man ₉ GlcNAc ₂ clusters of gp120	110
2	Inhibitory effects on HIV-1 RT	HIV-1 RT	13 nm	HIV-1 RT aptamers	116
3	Inhibitory effects against X4-HIV-1 and R5-HIV-1 isolates	HIV-1 isolates	3–3.6 nm	HSG _n (SO ₃ [−]) _m	125
4	Inhibited replication of HIV-1 by RAL	HIV-1	1.8 ± 0.32 nm	AuNP-TAMRA and AuNP-glucose	92
5	Mucosal permeation for EGNz was higher than manEGNz	gp120	10.2 nm	EGNz, manEGNz, EFV	117
6	Neuroprotection and neurodegeneration by galectin-1	Infiltrate leukocytes	19.1 nm	Au-Gal-1	124
7	Inhibition of antiviral activity and p24 antigen	p24	2–10 nm	AuNPs and pegylated AuNPs	100

(EFV) and AuNPs. Finally, the preparation of a thermosensitive gel loaded with niosomes was conducted to form a microbicide nanogel. Mucosal permeation in the case of EGNz (EFV- and AuNP-loaded niosomes) was higher than manEGNz (mannan-anchored EGNz). The drug susceptibility assay shows that when EFV: AuNPs are used reduction in p24 production is higher when compared to that with EFV alone. Using poloxamer 407 (PLR 407), hyaluronic acid (HA), and carrageenan (CGN), formulation of the thermosensitive gel was done which acted as a vehicle.¹¹⁷

HIV infection is preceded by activation of microglia which get infected after coming in direct contact with infected cells and/or viral proteins, contributing to the development of HAND (HIV associated neurocognitive disorders).¹²¹ Upregulation of the surface receptors, secretion of excitatory amino acids, chemokines, and proinflammatory cytokines, and generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) are all exhibited by the activated microglia and as a result of activation of these products other resident cells get activated in the CNS resulting in recruitment of inflammatory cells in the brain that leads to neuronal apoptosis and neurotoxicity.^{122,123} Galectin-1 is a β -galactoside-binding protein that performs an important role in neuroinflammation and has diverse biological activities. In the research done by Ravikumar Aalinkeel *et al.*, *in vitro* effects in CHME-5/HIV cells were evaluated *via* galectin-1 treatment. Moreover, the AuNP-galectin-1 (Au-Gal-1) nanocomplex was prepared. The chemotaxis assay was conducted to check the bioactivity of Au-Gal-1. Its bioavailability increased, activity enhanced, and showed limited cell migration in comparison to free galectin-1.¹²⁴

4.4 Dendronized anionic AuNPs for HIV-1 inhibition

AuNPs have been stabilized by using carbosilane dendrons containing a thiol functional group at the focal point and sulfonate moieties at the periphery. These anionic dendrons and AuNPs have been tested for HIV inhibition.¹²⁵ Dendrons and dendrimers have similar properties except for the shape, which in the case of dendrons is cone-shaped containing the dendron surface and focal point. Therefore, the transformation of multivalency into nanosystems can be achieved as an advantage through dendronization.¹²⁶ Two pathways were used to prepare AuNPs – through place exchange reaction and by direct reaction of dendrons with a gold precursor in the

presence of a reducing agent in water. AuNP systems of the type Au@($\text{SG}_n(\text{SO}_3^-)_m$) were generated and tested for toxicity and inhibitory capabilities. Variable sizes of the gold core (Table 3) were determined through TEM. The luciferase-based assay was employed to evaluate the effect of AuNPs and dendrons in TZM.bl cells against X4-HIV-1 and R5-HIV-1 isolates *in vitro*. X4-HIV-1 was partially affected by free dendrons in comparison to anionic AuNPs which exhibited an inhibitory effect against both HIV isolates.

Cytotoxicity. AuNPs and dendrons have been tested to determine their cytotoxicity in TZM.bl cells through the MTT assay. The tests revealed that conjugation with NPs decreased the toxicity for the third generation-derivative, whereas the toxicity for first and second-generation dendrons was lower without conjugation in comparison to that measured in NPs.¹²⁵

5. Conclusion and future outlook

An even brighter future is expected for gold nanomaterial applications in this area with the aim of targeting only tumor cells and having limited detrimental effects on normal human cells. Gold nanotechnology-based gene therapy is emerging as one of the promising alternative approaches for future HIV/AIDS treatment by insertion of a gene into the cell, which interferes with viral infection or replication. Additionally, other nucleic acid-based compounds such as DNA, siRNA, RNA decoys, ribozymes and aptamers or protein-based agents (fusion inhibitors and zinc-finger nucleases) can also be used to interfere with viral replication. In order for the genes to be inserted, there is a need for a vector system that is non-toxic, biodegradable, and penetrable to host cells. Significantly, viral vectors for HIV/AIDS gene therapy have long been tried for application in several clinical trials while still being sceptic about their toxicity, immunogenicity, insertion mutagenesis and viability. Hence, this has provoked investigation of non-viral vector systems, where gold nanoparticles can play a promising platform for their biomedical applications in future. Future developments in gene-derived toxicogenomic research may create new methods for assessing toxic effects of targeted nanomedicines in biological fields. In addition, the upcoming concept of green methods of synthesis of gold nanoparticles will also play a major role in this biomedical field.

Abbreviations

HIV	Human immunodeficiency virus	CNS	Central nervous system
AIDS	Acquired immunodeficiency syndrome	AuNP-TAMRA	Gold nanoparticle-tetramethylrhodamine
WHO	World health organization	HBMECs	Human brain microendothelial cell
ELISA	Enzyme-linked immune sorbent assay	PBMCs	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction	iDCs	Immature dendritic cells
QCM	Quartz crystal microbalance	CLRs	C-type lectin receptors
HAART	Highly affective antiretroviral therapy	ICAM	Intercellular adhesion molecule
AuNPs	Gold nanoparticles	FC	Flow cytometry
ART	Antiretroviral therapy	CLSM	Confocal laser scanning microscopy
BBB	Blood–brain barrier	PEG	Polyethylene glycol
ERGO	Electrochemically reduced graphene oxide	PDDAC	Poly(diallyldimethylammonium chloride)
ICPMS	Inductively coupled plasma mass spectrometry	PEI	Polyethyleneimine
BA	Biotin–streptavidin	pEGFP	Plasmid-enhanced green fluorescent protein
LF	Lateral flow	TEM	Transmission electron microscopy
POC	Point of care	MDDCs	Monocyte-derived dendritic cells
SERS	Surface-enhanced Raman scattering	APCs	Antigen presenting cells
Apts	Aptamers	STD-NMR	Saturation transfer difference NMR
TAT	Transactivator	IC	Inhibitory concentration
RNase	Ribonuclease	SELEX	Systematic evolution of ligands by exponential enrichment
RT	Reverse transcriptase	rDNA	Random DNA
ssDNA	Single-stranded DNA	RAL	Modified raltegravir molecule
ssRNA	Single stranded RNA	EGNz	EFV- and AuNP-loaded niosomes
PAGE	Polyacrylamide gel electrophoresis	MPS	Mononuclear phagocyte system
CHIT	Chitosan	EFV	Efavirenz
PGE	Pencil graphite electrode	CGN	Carrageenan
DEF	Deferiprone	HA	Haluronic acid
EIS	Electrochemical impedimetric	PLR	Poloxamer
R_{CT}	Charge transfer resistance	HAND	HIV associated neurocognitive disorders
HRP	Horse radish peroxidase	ROS	Reactive oxygen species
LSPR	Localized surface plasmon resonance	RNS	Reactive nitrogen species
VLPs	Virus like particles	Ag	Antigens
ITO	Indium tin oxide	P-RGO@Au@Ru-SiO ₂	Gold nanoparticle-decorated graphene (PRGO@Au) and Ru(bpy) ₃ ²⁺ -doped silica (Ru-SiO ₂) nanoparticles
LFA	Lateral flow assay	PLR 407	Poloxamer407
hFTH	Human ferritin heavy chain	RES	Reticuloendothelial system
EDX	X-ray spectroscopy		
TAR	Transactivation response element		
LIA	Laser light scattering immunoassay		
DLS	Dynamic light scattering		
CMC	Carboxymethylcellulose		
IC	Immunochromatographic		
PIs	Protease inhibitors		
PR	Viral protease		
LPV	Lopinavir		
CGC	Colloidal gold nanoparticles		
MWCNTs	Multi-walled carbon nanotubes		
AEP	Acetone-extracted propolis		
ECL	Electrochemiluminescence		
NPs	Nanoparticles		
BSA	Bovine serum albumin		
MUA	Mercaptoundecanoic acid		
MGITC	Malachite green isothiocyanate		
CTAB	Cetyltrimethylammonium bromide		

Conflicts of interest

The authors declare no conflict of interest, financial or otherwise.

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