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Diagnosis and analysis of HIV-1 with different methods

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Abstract. Early detection and continuous monitoring of human immunodeficiency virus type 1 (HIV-1) infection are crucial for effective diagnosis and treatment. To ensure successful early ART application onto patients in early stages, methods of HIV-1 detection in premature HIV accumulation stages are important to prevent the accumulation of HIV-1 viral molecules after replication. At the same time, biomarkers such as CARD8 and TAT proteins are an important the early identification and monitoring mechanism of HIV-1 infection. These biomarkers are the building blocks of a successful biosensor and they can provide key insights into disease progression and treatment efficacy. This research provides an extensive review of HIV-1 biomarkers and potential carbon-based biosensing mediums such as carbon dot sensors and carbon nanotube sensors in order to detect HIV-1 viral detection after surface modification with a focus on their implications for early detection and monitoring.

Keywords: HIV-1, biomarkers, detection.

1. Introduction

Over the past decade, the diagnosis of severe retroviruses has drastically increased. Retroviruses, typically referring to a group of viruses that initiates enzyme reverse transcriptase to reversely damage the host cell's DNA by establishing a complementary DNA copy of the viral RNA genome which disables the functionalities of targeted immune cells. An example of such a virus is the human immunodeficiency virus (HIV). Approximately 38.4 million people across the globe are infected with HIV [1], bringing cumulative death of 650,000 annual deaths in 2021 [2]. While these viruses can inflict substantial harm on human physiology, early treatment methods have demonstrated efficacy in mitigating the severity and progression of HIV-1. Thus, the development of efficient, reliable, and effective early diagnostic techniques is of grave importance in combating these complex illnesses.

Achieving early detection of retroviruses necessitates a comprehensive understanding of the sensing mechanisms that needs to be utilized. The conventional approach for detection facilitates via the use of ELISA immune assays, it is experimented that with HIV-1 viral infection, HIV-IgA complexed form. The existing research presented findings via commercial ELISA IgA test kits on HIV-1 on infants born, results showed the formation of HIV-IgA complexs within 53 out of 64 of the infected infants achieving a high sensitivity (95%) and selectivity (100%), and accurately revealed all infected infants. ELISA assays utilize fixated antibodies on the surface and would change in its color when an antigen-antibody complex form as antibodies attaches to HIV viral antigens such as IgM or IgG Abs. Although ELISA assays provide highly sensitive and low noise results, the detection via ELISA must be within a window period. For direct ELISA assays, the condition of when the human

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immune system is unable to produce HIV antibodies should be achieved so that there would be no competition of the antigen-antibody complex. Additionally, such methodologies can be time-consuming and require laboratory equipment and labor intensive.

Another wide spread practice of HIV sensing is through PCR technologies, with fine-tuned primers, it easy to detect HIV strains and viral caused proteins within patients' blood. The results showed PCR's effectiveness in HIV-1 detection in infant blood samples [3]. Sensitivity of the PCR technology showed the sensitivity of 100% among the 83 specimens 50 are identified as HIV-1 negative, PCR identified 10 out of 10 patient's specimens. However, the variability in the HIV-1's viral RNA attains too many mutations which varies on the selection of PCR primers thus has to be changed frequently. Additionally, PCR is also labor extensive and requires extensive time to collect patients results. Therefore, it is important to analyze alternative methods that are less time dependent and more cost-effective, ensuring expedited diagnosis of early-stage HIV infections.

Alternatively, to the time-consuming immuno-assays and PCR which all require the regeneration of antibodies or the incubation of viral RNA in order to be performed. Optical biosensors which utilizes florescence can detect a target analyte with faster sensing of the viral molecule whilst attaining high sensitivity and low background noise. However, such sensors require extensive research and specific biomarker molecules in order to achieve successful early HIV detection.

The research focused on the early diagnosis of HIV by assessing various biomarkers that utilizes its optical properties which would ultimately resolve the absence of early HIV detection by using multiple biomarkers as the analyte. The viral infection of HIV would initiate many intracellular and extracellular responses. To attain and successfully sense the increase in the quantity of these biological molecules would ensure the early identification of HIV.

2. HIV-1 analysis

As briefly discussed in the introduction, the retrovirus employs a unique pathway for its transmission method by using RNA reverse transcriptase, which reversely transcribes RNA information of the virus to host cells. In the case of HIV, the retrovirus attacks and destroys CD4-attaining immune cells, causing a deficient immune system.

There are two types of HIV. The difference between the two variants, HIV-1 and HIV-2 resides in the surface protein gp120, the docking glycoprotein that binds to the receptors of the CD4 cells. Both types exert similar traits and effects but vary in severity and transmissibility. HIV-1 has higher likelihood of perinatal transmission than HIV-2. The pathogenesis of HIV-1 also shows an increasing rate of conversion to AIDS. Lastly, HIV-2 seems confined to Africa, whereas HIV-1 is transmitted worldwide [4].

The morphology of HIV is similar to regular viruses, consisting of a dense, cone-shaped p24 icosahedral protein capsid enveloped by a lipid membrane layer. The virus's RNA genome carries two identical copies of itself and associates with intrinsic enzymes. Understanding the mechanism of how such retroviruses infects CD4 cells is a crucial factor in early biosensing, as the target analyte could be selected to initiate the sensing process.

HIV attains its infection cycle consists of seven processes. At the binding stage, the viral trimeric complex, composed of glycoproteins gp42 and gp120, binds towards the CD4 receptor of the immune cell. In order for the fusion between the virus and the target cell. The viral complex also has to bind to various co-receptors [4]. These signaling ligands can activate metabolic pathways to proliferate cell growth and replication. The two most signature co-receptors involved in this process are CXCR-4 and CCR5, which exhibit varying pathways which alter the tropism of HIV [5]. CCR5 is a co-receptor typically found in macrophages, dendric cells and CD4-t helper cells. The CCR5-CD4-HIV complex generates M(macrophage)-tropic HIV viral infection. However, the chemokines RANTES, MIP-a and MIP-b, which are associated with CCR5, can suppress HIV as they compete with the virus for the binding of CCR5 [5]. Similar suppression signatures also occur with the CXCR-4 receptor, which initiates a T-tropic HIV infection. As gp120 binds with CD4 and CXCR-4, the a-chemokine SDF-1 inhibits the replication of these t-tropic HIV viruses. The stabilized "docking" of the HIV viral allows

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it to proceed into further fusion via the gp41 protein, which fuses the HIV envelope with the host cell's membrane. The latter stages of the pathogenesis of HIV include the fusion of the viral particle with the host cell's membrane via various glycoprotein receptors, the reverse transcription of the RNA viral genome into DNA, and then finally, producing excessive daughter viral components, which exits the host cell, thus allowing an efficient infection of the virus through the lymphatic system where viral information can be easily bound to different CD4-attaining cells.

3. HIV-1 protein biomarkers

3.1. CARD8 proteins

Caspase recruitment domain family member 8 (CARD8) is a protein which is stimulated as a type-1 HIV virus infects CD4+ cells [6]. The caspase recruitment domain family belongs to a larger group of death domain (DD) family which are a type of TLRs which triggers cellular necrosis and apoptosis. When CARD proteins are activated, it initiates the activities of caspase enzyme. Aside from HIV, CARD member proteins also associate with other diseases. Caspase itself is cysteine protease which activates through the cascade of enzymic activity leading to the apoptosis of the cell.

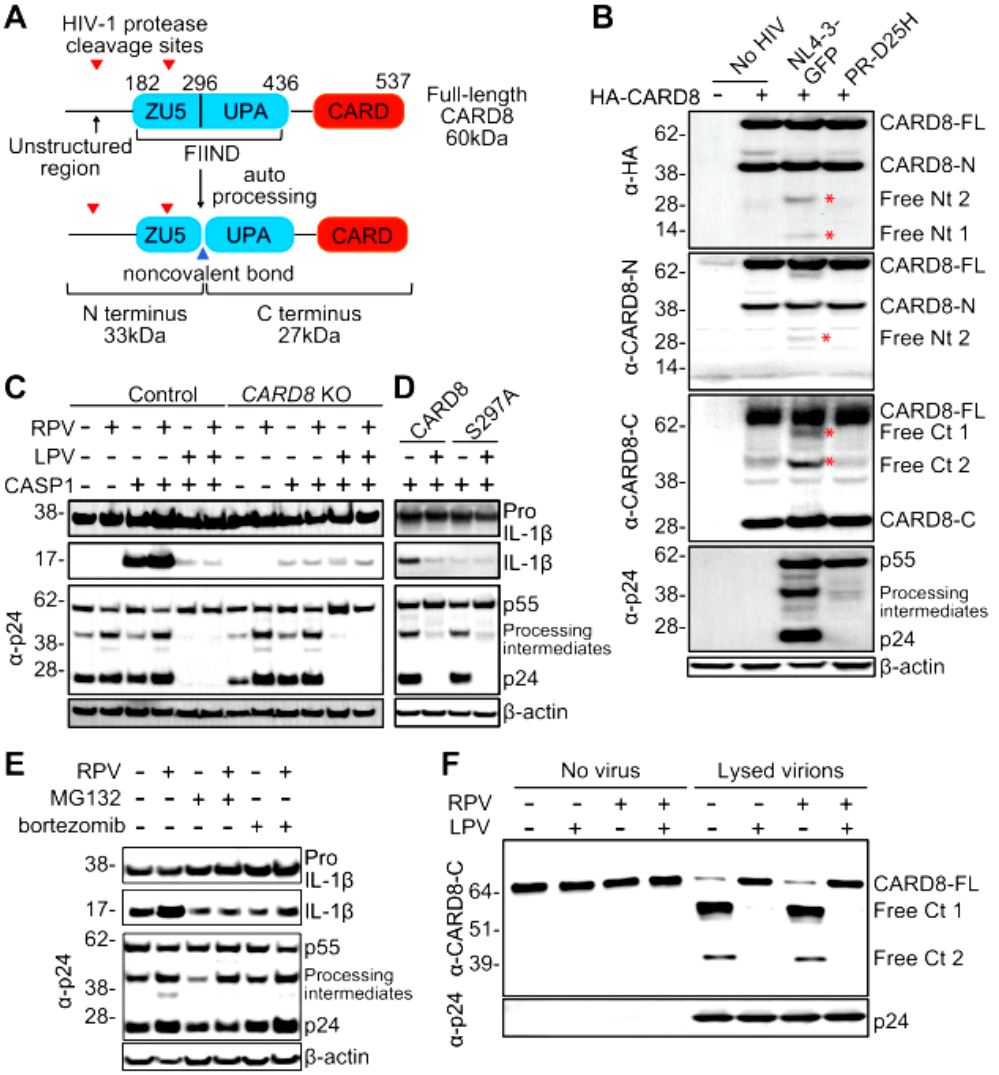

Figure 1. Application of CARD8 inflammasome for detection of HIV-1 protease activity [6, https://www.science.org/doi/abs/10.1126/science.abe1707].

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CARD8 more specifically can act as a sensor for type-1 HIV infections. CARD8 protein is an inflammasome biomarker of the HIV viral protease activity [6, 7], as shown in Figure 1. During the experiment, HA-CARD8S297A was immunoprecipitated and incubated with the lysed HIV-1 particles. As the active protease cleaves the protein. Cleavages that occur in the N-terminus triggers the proteins response which then triggers the CARD-8 dependent pyroptosis of the infected macrophages. From the conclusion of the experiment, the CARD8 inflasome's initiation which triggers the immune cascade generates bulk CARD-, ASC-, CASPI-, THP1- cells, which are also molecules that promotes the cell's apoptosis. The bulk increase in the cells CARD8 generated response can therefore be sensed.

CARD8 sensing can be a potential effective diagnostic to early-stage HIV-1 infection as CARD8 proteins are highly expressed in blood and lymphoid tissues. The lymphoid tissues, where HIV-1 infections are highly expressed and would allow fast and efficient sensing of the HIV viral molecule.

3.2. TAT protein

The twin arginine translocation protein is a trans-activator of transcription protein. The TAT HIV proteins are overly expressed as it can enhance the efficiency of the viral transcription. TAT protein, when initiated within the patient's body, enhances the reverse transcription rate of the HIV RNA. This enables the protein to become a highly noted protein as it will accumulate on the presence of HIV-1 viral information at the very start of a HIV viral infection.

The increase in the quantity of HIV viruses would result in the elongation of RNA polymerase. It was first proposed that TAT receipts CDK9 and Cyclin T1 to TAR RNA at the 5' viral transcripts, thereby elongating carboxyl terminal domain of the RNAPII (RNA polymerase II) via the phosphorylation of CDK9, promoting the reverse transcription of the viral RNA. However, after extensive experiments, it is found to be that TAT operates in a more complex manner: Further transcription research identified two multiprotein complexes, NELF and DSIF which associates with the RNA polymerase complex to inhibit the activation of the transcription.

4. Optical biosensors

For a higher rate of biomarker's detection. The uses of PCR after lysising viral material methods tend to be extremely time consuming by needing DNA restructure time and incubation time, thus making it an inefficient way of sensing the biomarker molecules especially in mobile and rural areas with small opportunity to access laboratories. This makes electrochemical sensors such as carbon-based sensors become a good consideration due to its excellent optical and electric conductible properties. Carbon dots and other larger carbon nanotubes all have the conditions needed to be a sensitive and efficient biosensor.

4.1. Carbon dots-based biosensors

Carbon dots (CDs) are carbon-based nanoparticles averaging in 10 nm to 100 nm in diameter and are manufactured as biosensing molecules. The creation of such particles is due to the inability of utilizing normal macroscopic carbon materials to exhibit fluorescence and higher sensitivity in conducting electricity. Thereby, smaller and better broken-down molecules such as carbon dots are synthesized as biosensing molecules.

The understanding of synthesizing approaches is crucial for researchers to effectively and efficiently produce morphologically similar CDs which can give off similar electron transfers and relatively similar modifications to its surfaces. The synthesizing strategies of carbon quantum dots are separated into two approaches: 'top-down' and 'bottom-up' strategies. Within the 'top-down' strategy, more significant carbon-based precursors are broken down into smaller particles. The top-down approach usually forms larger-sized carbon dots, which are graphene quantum dots as it averages around 100 nm in diameter. Graphene and fullerenes are broken down within set conditions via chemical exfoliation. This particular method applies precursor carbon materials with strong acids or

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oxidizing agents to initiate the breaking down of more extensive macroscopic materials such as carbon fibres, graphene oxide or carbon nanotubes.

The 'bottom-up' approach involves the polymerization and carbonization of small carbon molecule precursors such as citric acid and glycerol to 'build up' a molecule. The 'bottom-up approach is usually used to produce smaller carbon dot molecules via pyrolysis. These smaller-sized carbon dots are usually carbon quantum dots that average 10 nm in diameter and obtain a spherical shape. During microwave pyrolysis, the microwave exerts uniform radiation on the carbon precursors to form CDs. The production of such small sized carbon nanodots allow the molecules to persist different florescent properties, thereby allowing the electron transfer intermediates to be substituted with similar properties to ensure top reliability.

For the detection of HIV particles. The carbon dots can use its FRET and IFE properties in order to sense the HIV-1's gene detection. The fluorescence resonance energy transfer (FRET) allows the transformation of nonradiative energy between an excited donor and acceptor via long-range dipole-dipole interactions. During carbon dot based biosensing, CDs will act as the donor of electrons to the biomarkers. If CDs overlaps the biomarker's analyte's absorption spectrum, FRET occurs causing optical variations. However, FRET requires highly matching absorption spectrum of the target analyte with the tailored CDs. Which requires further modification of the CDs to enable easier FRET strategies which ensures high sensitivity and selectivity toward the target analyte. Secondly, inner filter effect (IFE) is also a property that carbon dots can utilize when sensing the biomarkers.it is the re-absorption of the excitation or emission light from the CDs. Which stands for the variable for whether the carbon dot's PL is quenched or not. Unlike FRET, the IFE biosensing approach in carbon dots aims at a more flexible and straightforward without the link of absorber with fluorescent. For the application of IFE based biosensing, the strategy changes when meeting different criteria [8]. When absorbance spectrum of the biomarker analyte overlaps effectively with the CDs excitation or emission spectrum, the strategy can be continued without any changes, enabling the inner filter effect. However, if the absorption does not overlap, an absorber will first be stoichiometrically reacted to the target analytes, therefore allowing the inner filter effect to be initiated. Additionally, aside from being an excellent sensor material. CDs has also shown a new frontier in the inhibition of HIV viruses. CDs act as an inhibitor to prevent the binding of the gp120 protein with CD4 and the co-receptors, which further adds the functionalities of carbon dot molecules.

It is suggested due to CDs excellent compatibility and highly modifiable nature that, such nanomaterial-based biosensors could partake in viral infections such as HIV retroviruses. The large surface area to volume ratio and high functionalization within GQDs (a subgroup of CDs) enables it to become a signal amplifier within an electrochemical biosensor. The determination of the electrical signal is the main mechanisms behind electrochemical biosensors where a particular analyte will produce the specific reaction with the modified surfaces of CDs with the addition of different functional groups.

The results shown GQDs efficiency in Hepatitis B virus DNA (HBV-DNA) detection [9]. The GQDs were first synthesized via pyrolysis process using citric acid. The probe of the sensor shows the electron transfer from the prepared GQD electrodes to the $K_3[Fe(CN)_6]$, enabling the recording of the electrical signal. When there is the presence of HBV-pDNA results a low value peak of the current due to the electrostatic repulsion between the GQD and the target pDNA. This strategy proves to be efficient within a 10 to 500 nM linear DNA range, enabling it to become extremely sensitive and inexpensive. However, such methods in detecting viral DNA may vary with HIV-1 detection methods. This is due to the difference in stability between DNA and RNA molecules: HIV-1 only attains RNA instructions that reversely transcribes into DNA that prelongs within host cells, therefore alternative strategies need to be analyzed based on existing methodologies.

4.2. Carbon nanotube-based biosensors

Aside from carbon monomer-based sensing mechanisms. Larger carbon nanotubes also present good fluorescent properties. Harvey et al. modified carbon nanotubes which allowed direct sense the virus'

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RNA or viral oligonucleotides without the amplification or incubation of fluid samples [10], as shown in Figure 2. Such functionalities allow the carbon nanotube biosensors have more efficient sensing and facile detection method as it also involves optical changes. Carbon nanotubes' photobleaching resistance also enables it to become a stable and durable product.

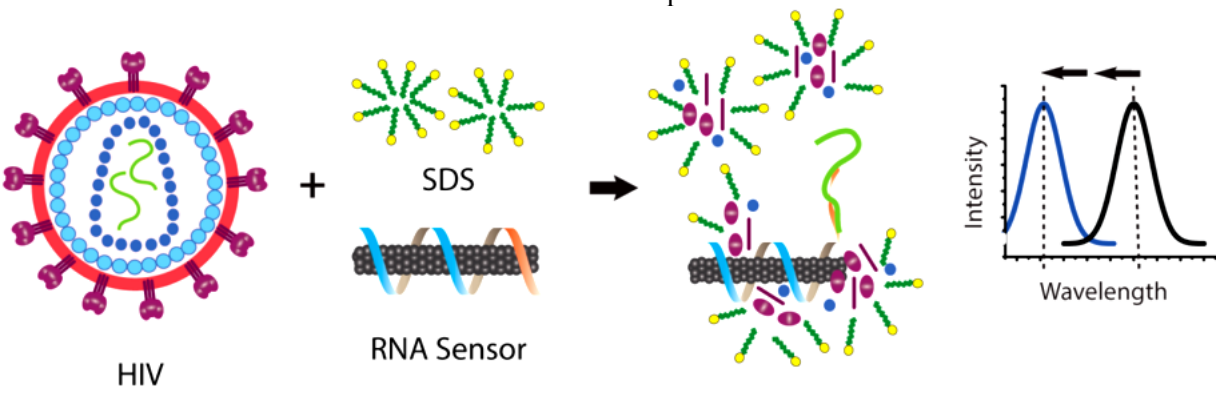

Figure 2. HIV detection with carbon nanotube-based sensing method [10, https://doi.org/10.1021/acssensors.9b00025].

As it is also a carbon-based material, similarly to CDs, can be modified via the addition of functional groups. Since oligonucleotides are small microRNAs which is found in trace amounts, it is usually hard to sense such molecules of an early HIV infection. However, carbon nanotubes, whilst detecting for microRNAs, with simple buffer conditions can attain a shit in the emission of the wavelength that changes the optical properties with the modification of sodium dodecylbenzenesulfonate (SDBS) [11]. Within the experiment, carbon nanotube-based biosensor was used for sensing miR-19 microRNA sequence. Within buffer only solutions, the target miR-19 DNA causes a blue shift in the nanotube emission. However, by adding the fetal bovine serum (FBS), a cell culturing media, creates an abrogated response of the change in wavelength.

To capture and understand the optical changes, a two-dimensional photo luminescence excitation spectroscopy is utilized to assay the changes of the carbon nanotubes within the FBS and miRNA strands. With the increasing in the concentration of FBS, it promoted the red shifting of the wavelength spectroscopy. It is explained that the nanotube emissions respond to the changes in the dielectric environment, causing the close association of the proteins of the serum with the nanotubes, thus causing the coating of the proteins in the nanotube's bare regions by electrostatically interacting with the phosphate backbones of the polynucleotides. Although the single walled carbon tubes are prone to experience wavelength distortion. It is later hypothesized that the addition of sodium dodecyl sulphate can suspend the nanotubes. Allowing a steady optical variation; creating the blue shift. Thus, insuring the specificity and accuracy of the nanotube, which also allowing an efficient point-of-care diagnosis.

5. Conclusion

The dissertation lead insights to the most recent advances in biosensing techniques in the detection of early viral HIV-1 infections by utilizing direct and indirect sensing mechanisms that track the viral RNA or various biomarkers that accumulate with the activity of the reverse transcription. By ensuring a fast, high-sensitivity and bio-compatible sensor ensures optimized effeminacy for point of care, early staged HIV-1 patients. This then leads to the earlier detection of HIV-1 of patients at the same time ridding the impracticability of PCR or ELISA induced sensing strategies. The earlier detection brought by carbon-based sensors or alternative biosensors, with earlier HIV-1 sensing, patients practicing antiretroviral therapy (ART) attains a higher chance of succeeding in their treatments which suppresses HIV counts within a patient. Therefore, novel biosensors that utilizes sensing molecules based on carbon derivatives provide us alternative yet efficient ways to detect targeted HIV-1 viral molecules that is surpasses traditional detection methods such as ELISA tests or PCR tests. Allowing patients to

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gain access to earlier ART treatments which additionally benefits the treatment of co-infections such as mycobacterium tuberculosis or HBV diseases. However, it has to be acknowledged that alternative treatment methods also need to explored to add on to earlier detection methods. Although ART ensures the prevention of further HIV spread via inhibiting the life processes of the HIV-1 viral molecules. Its long term and continuous costs for patients at times can discourage treatment in poorly developed countries with averaging annual prices of 880$ per USA patient. Alternative, fast-curing treatment methods, along with more efficient, real-time diagnostic could potentially achieve the control and eradication of such retroviral diseases.

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