NANOPLASMONIC BIOSENSING PLATFORM FOR MULTIPLE PATHOGEN DETECTION

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ABSTRACT

In this study, we present for the first time a reliable, fast, accurate and sensitive multiple pathogen detection platforms that can be adapted to the point-of-care (POC) in resource-constrained settings. Here, nanoplasmonic properties of metal nanoparticles are utilized to detect the captured viruses on the biosensing surface, and the platform also allows to report a quantitative viral load for HIV-infected anonymous discarded patient whole blood samples. The limit of detection covers the definition of treatment failure indicated by World Health Organization (WHO) (viral load (VL)>5,000 copies/mL) and the Department of Health and Human Services (DHHS) and AIDS Clinical Trials Group (ACTG) (VL>200 copies/mL).

KEYWORDS

Nanoplasmonic, pathogen detection assays, HIV, viral load, point-of-care.

INTRODUCTION

Infectious diseases have a global healthcare impact on our daily lives from airports to office spaces as determined in H1N1 and AIDS/HIV. For instance, AIDS/HIV, one of the most life threatening pandemic, has resulted over 25 million deaths since first case was observed. In the US, there are 1.2 million HIV-infected people and only 28% of the HIV-infected patients are suppressed with antiretroviral therapy (ART). For successful ART, HIV-1 viral load is commonly used to closely monitor the patient response to ARTs in developed countries. In developing countries, CD4+ T cell count and clinical symptoms are used to monitor ART following the World Health Organization (WHO) guidelines. To our knowledge, currently no rapid HIV viral load platform technology exists at the point-of-care (POC) settings. So far, the HIV/AIDS pandemic has caused over 25 million deaths and over 450,000 infants are infected through mother-to-child transmission (MTCT) annually. To reduce morbidity and improve quality of life for people living with HIV/AIDS, WHO is rapidly expanding access to ART in developing countries accounting for over 67% of HIV-1 infections. However, the expansion is significantly restricted by the lack of cost-effective POC viral load assays. Commercially available HIV viral load assays are expensive ($50-200 per test), instrument-dependent, and technically complex, which are not accessible to resource-constrained settings. On the other hand, recent studies have shown that CD4+ cell counting strategy cannot detect early virological failure, which allows for accumulation of drug-resistant strains in infected individuals and reduces the efficacy of first-line drugs. Thus, a rapid, inexpensive, and simple viral load test is urgently needed at the POC settings.

Label-free biodetection systems such as electrical detection platforms [1] have been recently used for the detection and diagnostics of infectious agents [2-5]. These platforms enable to detect multiple pathogens for medical diagnostics and drug development/treatment studies. These technologies also minimize the requirements for fluorescent/radioactive labeling and offer easy-to-use, inexpensive POC tests.

Metal nanoparticles have been used in optical biosensing platforms for clinical diagnostics [6]. Optical properties of these nanoparticles have a vital property to monitor the changes surrounding environment of nanoparticles, and their plasmonic properties can be also used as an alternative method to detect infectious pathogens/agents such as fungi, bacteria, and virus. However, there are several engineering and sampling challenges (*i.e.,* chemical and physical modifications and the requirement of sample preprocessing) in both production and clinical testing of metal nanoparticle-based detection methods. Here, we present for the first time HIV viral load quantification assay from unprocessed whole blood using a nanoplasmonic optical detection system (Figure 1).

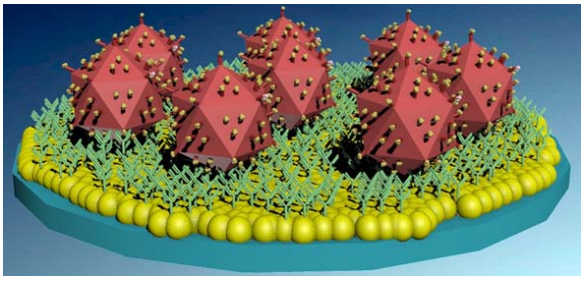

Figure 1: HIV capture on nanoplasmonic platform.

MATERIALS AND METHODS

To build nanoplasmonic platform, the polystyrene support material was first modified with amine-terminated groups to immobilize gold nanoparticles. Then, the

support material was activated using coupling reaction of 11-Mercaptoundecanoic acid (MUA) and N-Ethyl-N'-(3-dimethylaminopropyl) carbodiimide hydrochloride/N-hydroxysulfosuccinimide (EDC/NHS), and succinimide groups were generated to immobilize biorecognition elements. First, NeutrAvidin was covalently bound, and then, the polyclonal anti-gp120 antibodies were immobilized. Following each surface modification and activation, the biosensing surfaces were rinsed with 1x phosphate buffered saline (PBS) solution.

HIV culture samples were spiked in unprocessed whole blood, and a various concentrations of multiple HIV subtypes (A, B, C, D, E, G, and panel) spiked in whole blood were evaluated using the platform. Further, the platform was validated using HIV-infected anonymous discarded patient whole blood samples.

For analysis, each binding event was characterized with a noticed wavelength shift at the maximum extinction point of gold nanoparticles using Varioskan® Flash Spectral Scanning Multimode Readers, Thermo Scientific. The detection light beam area of the spectrometer was indicated to be 3.14 mm^2. The spectral resolution was 1 nm, and the intensity accuracy of the instrument with a fixed slit setting was 0.003 a.u. as the manufacturer indicated. The measuring mode was adjusted to scan the extinction changes from 400 nm to 700 nm.

To analyze the data, each binding event was subtracted from its own antibody immobilization step, and the shift was indicated as wavelength change ± standard error of the mean (SEM).

To visualize the captured HIV samples, scanning electron microscope (SEM) was used. Polystyrene support surfaces were used to assess the capture of HIV samples. After surface modifications were completed, HIV spiked in whole blood samples were applied to the biosensing surfaces, and then, the surfaces were cut by a glass cutter to visualize the capture of HIV using SEM imaging. SEM images were taken at 4.7 mm working distance and 4.00 kV accelerating voltage.

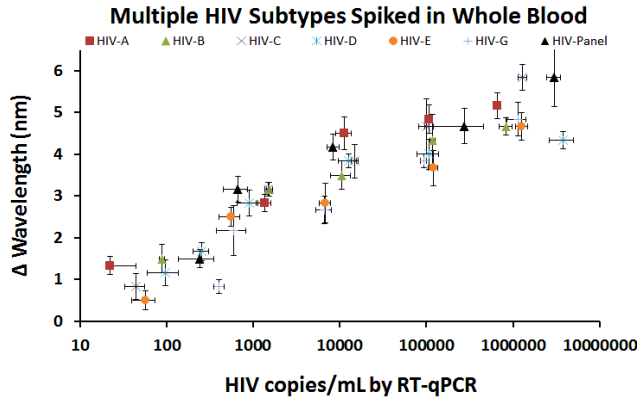

Figure 2: Validation of nanoplasmonic platform with multiple HIV subtypes (A, B, C, D, E, G, and panel) spiked in whole blood samples.

RESULTS AND DISCUSSIONS

To evaluate the system performance and limit-of-detection, we analyzed various concentrations of multiple HIV subtypes spiked in whole blood samples ranging from 50 to 10^6 copies/mL. HIV subtype A samples resulted in a 5.2 ± 0.4 nm wavelength shift when (6.5 ± 0.6) x 10^5 copies/mL was applied to the biosensing surface. The peak shift was observed to be 1.3 ± 0.2 nm when the lowest concentration (22 ± 20 copies/mL) was analyzed (Figure 2). (8.3 ± 1.3) x 10^5 copies/mL copies/mL of HIV subtype B samples caused a wavelength shift of 4.7 ± 0.5 nm. The peak shift decreased to 1.5 ± 0.5 nm, when 89 ± 6 copies/mL HIV concentration was evaluated (Figure 2). For sampling with HIV subtype C samples, (1.3 ± 0.2) x 10^6 copies/mL concentration was evaluated, and the peak shift was observed to be 5.8 ± 0.8 nm. At (1.5 ± 0.1) x 10^3 copies/mL concentration, 3.2 ± 0.5 nm wavelength shift was observed (Figure 2). (3.8 ± 1.2) x 10^6 copies/mL of HIV subtype D samples resulted in a wavelength shift of 4.3 ± 0.5 nm. At the lowest concentration ((8.9 ± 2.4) x 10^2 copies/mL), 1.3 ± 0.5 nm wavelength shift was observed (Figure 2). HIV subtype E samples were evaluated and observed that there was 4.7 ± 0.5 nm wavelength shift at (1.3 ± 0.2) x 10^6 copies/mL. The peak shift decreased to 0.5 ± 0.5 nm when the lowest concentration (57 ± 17 copies/mL) was used (Figure 2). For sampling with HIV subtype G, (1.1 ± 0.3) x 10^6 copies/mL concentration was evaluated, and the peak shift was observed to be 4.8 ± 0.5 nm. At 404 ± 54 copies/mL concentration, 0.8 ± 0.5 nm wavelength shift was observed (Figure 2). HIV subtype panel samples were also evaluated and observed that there was 5.8 ± 0.7 nm wavelength shift at (2.9 ± 0.5) x 10^6 copies/mL. The peak shift decreased to 1.5 ± 0.5 nm when the lowest concentration (245 ± 101 copies/mL) was used (Figure 2).

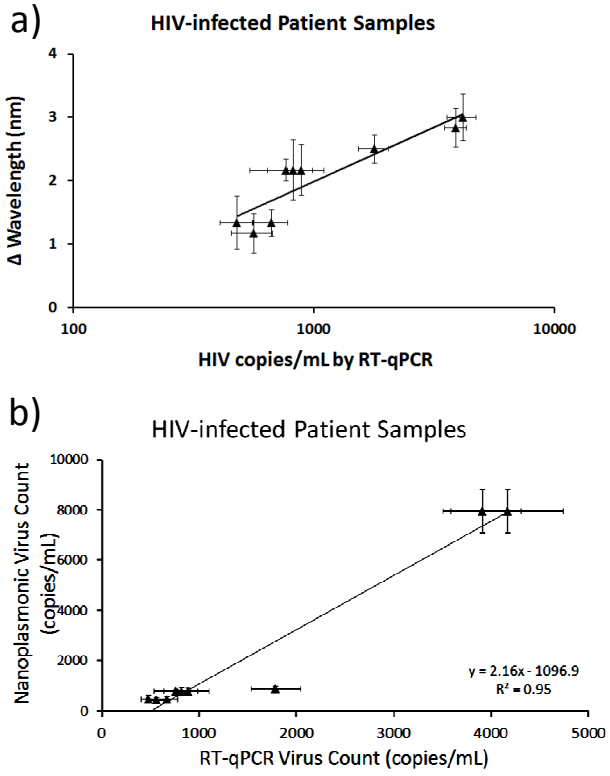

Figure 3: Validation of nanoplasmonic platform with HIV-infected patient samples. a) Wavelength shifts for HIV patient samples. b) Quantitative results for HIV patient samples.

Additionally, the nanoplasmonic paltform was evaluated with 9 HIV-infected anonymous discarded patient whole blood samples (Figure 3a). In the presence of patient samples, the highest peak shift was observed to be 3.0 ± 0.5 nm at 4169 ± 578 copies/mL (Figure 3a). The peak shift for 481 ± 73 copies/mL HIV viral load was observed to be 1.3 ± 0.5 nm (Figure 3a).

To evaluate quantitative detection, the standard curve was obtained from HIV spiked samples using the wavelength shifts and the HIV viral load obtained by RT-qPCR. The patient samples were quantified using this standard curve. The platform demonstrated a viral load ranging from 414 ± 109 copies/mL to 7966 ± 864 copies/mL in HIV-infected patient samples. RT-qPCR indicated a viral load ranging from 481 ± 73 copies/mL to 4169 ± 578 copies/mL in HIV-infected patient samples (Figure 3b).

Additionally, the captured viruses were presented using SEM imaging (Figure 4). The SEM analysis demonstrated that captured viruses at multiple locations did not show any aggregation.

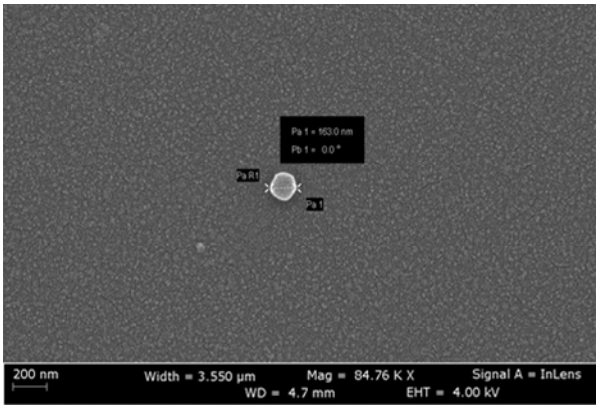

Figure 4: SEM image of the captured HIV on nanoplasmonic platform.

Additionally, the presented platform can be potentially used for infant testing where HIV status cannot be monitored by CD4+ counting, and nucleic acid amplification assays [7-9]. Another potential application can be to monitor co-infections such as tuberculosis-HIV [10]. Further, this detection platform has multiple advantages over the current viral load assays such as RT-qPCR tests for both infant and adult HIV testing. Further, the presented nanoplasmonic technology can be potentially used broad range of infectious agents/pathogens in clinical microbiology and infectious disease laboratories. This platform also offers a reliable, accurate, inexpensive, time-effective and ease-to-use detection platform for life-threatening infectious diseases such as AIDS and tuberculosis. Different clinically relevant samples such as nasal swabs, bronchoalveolar lavages, and nasopharyngeal aspirates or exhaled breath can be used in addition to the blood samples for detection of multiple pathogens. On the other hand, this technology presents a versatile platform to capture and detect other pathogens by modifying the biosensing surface with well-described markers. The limit of detection of the platform presents that low viral concentrations in whole blood can be detected without any changes on the morphology of intact viruses. Further, this detection platform offers to be deployed into a multiplexed format that would have a significant role in biosensor design and engineering to selectively and specifically capture and detect multiple pathogens and their subtypes from patient samples.

CONCLUSION

Here, we demonstrated for the first time HIV viral load quantification assay using nanoplasmonic platform for multiple HIV subtypes (A, B, C, D, E, G, and panel) spiked in unprocessed whole blood and HIV-infected patient whole blood samples with a high sensitivity down to 50 HIV copies/mL. The presented platform technology offers a fast, reliable, sensitive, specific, accurate, label and fluorescence-free quantification assay for viruses from whole blood without any sample preparation. This platform also employs the selective and specific capture and detection of intact viruses using the highly specific anti-viral antibodies, and reports a viral load covering a broad range of clinically relevant concentrations. Additionally, the system demonstrates the detection of intact viruses from unprocessed whole blood to be feasible for diagnosis of viruses directly from patient samples. Theus, this work holds a promise to provide reliable, accurate, inexpensive, label-free, ease-to-use POC tests at resource-constrained settings.

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