NANOPLASMONIC BIOSENSING PLATFORM FOR MULTIPLE PATHOGEN DETECTION

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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

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.

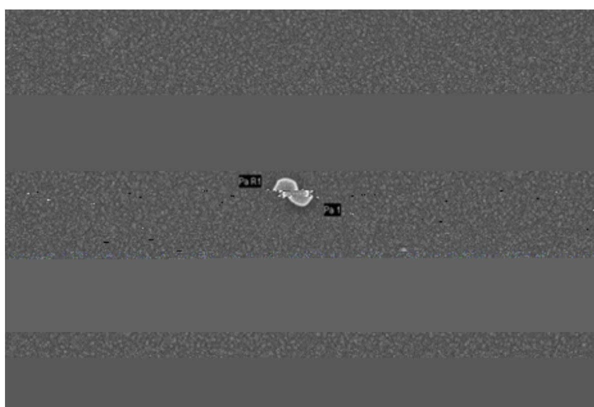

Figure 4: SEM image of the captured HIV on nanoplasmonic platform.

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

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