ACUTE HIV DETECTION BY VIRAL LYSATE IMPEDANCE SPECTROSCOPY ON A MICROCHIP

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ABSTRACT

Detecting acute Human Immunodeficiency Virus Human Immunodeficiency Virus (HIV-1) at the point-of-care (POC) is an unmet clinical need. Current POC detection methods such as dipsticks and OraQuick detect antibodies and not the intact virus. These methods are not effective due the low concentration of antibodies [1, 2]. Infected persons with acute HIV-1 can potentially transmit the infection because they are not aware of their disease [3, 4]. Rapid and inexpensive POC detection diagnostic tool to detect acute HIV-1 can potentially have a substantial effect on preventing HIV-1 transmission through people at high risk of HIV-1.

KEYWORDS

Acute HIV-1, microfluidics, biosensing, electrical sensing.

INTRODUCTION

Rapid, inexpensive, and portable biomicrosensors have broad applicability in clinical diagnosis and public health. Detecting infectious diseases including HIV-1 at resource-constrained settings is one of the promising and attractive applications. Specifically, detecting acute HIV-1 where viral replication and shedding (10^6-10^8 copies/mL) occur and the antibodies concentration is low and undetectable could have a substantial effect on the disease transmission prevention [2, 5]. Infected patients with acute HIV-1 can potentially have an important role in transmitting the disease since they might not be aware of their disease [3, 4]. For instance, statistically a significant ratio of the HIV-1 infected people in US (20% of the 1.2 million HIV infected people) is not aware of their HIV status. Further, and a majority of HIV infected population in the US (72%) is not suppressed with antiretroviral drugs [6]. These statistics clearly show the importance of developing a rapid, inexpensive, and disposable diagnostic tool for acute HIV-1 detection at the POC.

There are several commercially available RNA assays including Roche COBAS, Abbott Real Time, Siemens Versant, and bioMerieux NucliSens for HIV-1 viral load measurement in developed countries [7]. Other less complex assays such as P24 assay [8], the ExaVir RT viral load assay [9], and real time reverse transcription quantitative PCR (RT-qPCR) [1, 10] to measure the viral load are also available. However, these tests require expensive reagents and must be conducted by experienced operators at the field and consequently are not compatible with the requirements at resource-limited settings [7, 11, 12].

One of the attractive diagnostic methods used in biosensors is impedance spectroscopy of the biological sample. Electrical sensing in microfluidic devices on cells through Coulter counting [13], impedance spectroscopy [14], capacitance [15], and Dielectrophoresis (DEP) [16-19] have been utilized effectively for cells physical and electrical properties measurement and cellular manipulation [20]. Impedance change in a sample could have several reasons such as binding the target molecules to receptors [21], conductivity change of the medium by growth of cells [22], capturing bacterial cells with DEP [23, 24], and conductivity change because of ion concentration [25, 26].

To address the unmet clinical barrier for detecting acute HIV-1 at the POC, we have developed a microchip based on impedance measurement of the sample. This rapid (< 30 min), inexpensive (< $2), and portable HIV-1 is potentially compatible with the POC requirements and can offer a reliable detection platform for populations at high risk of HIV-1 infection and at doctor's office.

Viruses were captured on the surface of magnetic beads conjugated with gp120 antibodies and then detected using impedance spectroscopy of the viral lysate. During the viral lysis step, proteins, membrane phospholipids, capsid proteins, intracellular ions, retroviral enzymes, and nucleic acids are released into the bulk solution, which therefore change the electrical conductivity of the sample. Impedance magnitude of the bulk solution changes after viral lysate step and can be used as a sensitive marker for measuring the viral load. In this set up 1 μm diameter streptavidin-coated magnetic beads (Thermo Scientific, Rockford, IL) were mixed with biotinylated anti-gp120 antibodies (15 μg/mL) (Abcam®, Cambridge, MA) and conjugation occurred on a rotator (30 rpm) at 4°C for 2 hours. One key point in this detection process is removing the highly electrical conductive media after

virus capture. Samples were washed four times with an electrically low conductive solution (20% glycerol in grade water). For virus lysis step we used 1% Triton x-100 detergent (Sigma-Aldrich® St Louis, MO).

This electrical biosensing method demonstrates for the first time the ability to detect viruses at virus concentrations that occur at the acute stage of HIV-1 infection using viral lysate impedance spectroscopy in a rapid, simple, and inexpensive fashion. This label-free electrical sensing method of pathogen detection using impedance spectroscopy of pathogen lysate constitutes a broadly applicable platform for detection of other viral and bacterial pathogens in infectious diseases with reasonably well-described biomarkers including herpes, influenza, hepatitis, pox, malaria, and tuberculosis.

RESULST

We have tested the microchip with various HIV-1 subtypes at clinically relevant virus concentrations. HIV-1 subtypes (A, B, C, D, E, G, and panel (A, B, C, D, and circulating recombinant forms, CRF01_AE and CRF02_AG)) were used at concentrations of 1.7×10^8 (subtype A), 1.2×10^8 (subtype B), 1.2×10^8 (subtype C), 2.9×10^8 (subtype D), 8.4×10^8 (subtype E), 6.5×10^8 (subtype G), and 1.5×10^9 (panel) copies/mL in cultured media. The viruses were mixed with streptavidin-coated magnetic beads conjugated with biotinylated anti-gp120 antibodies. The samples were incubated at room temperature on a rotator (15 rpm) for half an hour. Control samples were prepared by adding viral-free DPBS solution into the streptavidin-coated magnetic beads conjugated with anti-gp120 antibodies. Samples were washed with an electrically low conductive solution (20% glycerol in water) 4 times to remove the electrically conductive background. The captured viruses were then lysed using Triton X-100, which is an electrically low conductive solution as well.

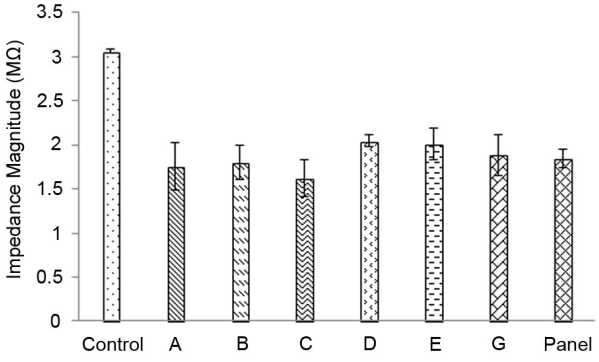

Figure 1. *Impedance magnitude of viral lysate samples of multiple HIV-1 suptypes including A, B, C, D, E, G, and panel as well as the impedance magnitude of control samples. The viral loads of 1.7×10^8, 1.2×10^8, 1.2×10^8, 2.9×10^8, 8.39×10^8, 6.5×10^8, and 1.5×10^9 copies/mL were measured for subtypes A, B, C, D, E, G, and the panel, respectively. The experiments were repeated 6 times and the error bars indicate the standard error of the mean value for the impedance magnitude.*

The viral lysate samples were then introduced into a two-rail electrode microchip (500 µm width and 2 mm spacing) for impedance measurement. Our experimental results represent the ability of the platform to detect multiple subtypes of HIV-1 (A, B, C, D, E, G, and panel of HIV subtypes) in a clinically relevant virus concentration (10^6-10^8 copies/mL) that appear at the acute stage (Fig. 1). Figure 1 shows the impedance magnitude of the viral lysate of HIV-1 subtypes A, B, C, D, E, G, and panel at 1,000 Hz and 1 V. The impedance magnitude of the viral lysate samples was significantly lower than the impedance magnitude of the control sample without virus (n=6, $p<0.05$). This method of virus detection is HIV-1 subtype independent as the impedance magnitude of the viral lysate samples for different subtypes were not statistically different compared to each other (n=6, $p>0.05$).

We have also been able to detect HIV-1 samples at concentration of 1 x 10^6 copies/mL by increasing the sample volume to 5 mL using the same amount of magnetic beads we used in smaller volume samples (100 µL). 5mL HIV-1 subtype C was mixed with 100 µL conjugated magnetic beads with anti-gp120 antibodies and incubated for a longer period of time (1 hour) with respect to the incubation time used for smaller samples (half an hour). This helped the magnetic beads to capture more viruses in a larger sample volume. Figure 2 shows the impedance magnitude spectroscopy of the viral lysate sample after 4 times washing and virus lysis step. The impedance magnitude of the samples decreased significantly with respect to the control samples. Control samples were conjugated magnetic beads with anti-gp120 antibodies suspended in DPBS solution without viruses. These results show the ability of the microchip to detect acute HIV-1 at clinically relevant concentrations (10^6-10^8 copies/mL).

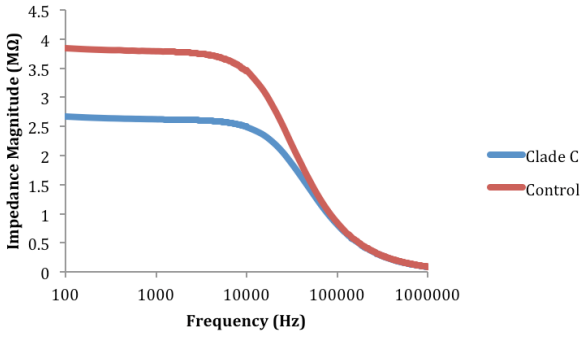

Figure 2. *Impedance magnitude spectra of HIV-1 clade C viral lysate sample at the concentration of 10^6 copies/mL.*

We have also tested the specificity of the microchip to detect HIV-1 in presence of Epstein-Barr Virus (EBV) as a model virus in the system. HIV-1 subtype B was mixed with EBV and conjugated magnetic beads with anti-gp120 antibodies were added into the mixed solution. The impedance magnitude spectra of the viral lysate samples with HIV-1, EBV, and mixture of HIV-1 and EBV was measured and is shown in Figure 3. The impedance

magnitude of samples with just EBV was not significantly different compared to the control samples. The impedance magnitude of the mixture of HIV-1 and EBV samples was significantly different than the impedance magnitude of the control samples as well as only EBV samples. However, the impedance magnitude of the mixture of HIV-1 an EBV samples was not significantly different than the impedance magnitude of only HIV-1 samples. These results indicate that the microchip is specific in capturing HIV-1 in the presence of another type of virus.

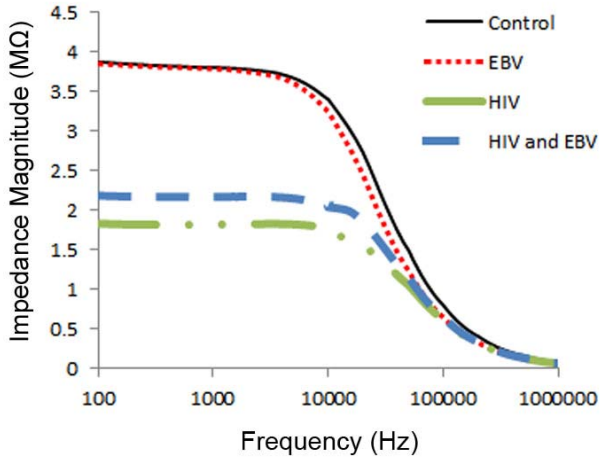

Figure 3. *Impedance magnitude spectra of viral lysate samples with HIV-1 subtype B, EBV, and mixture of HIV and EBV at concentration of 10^8 copies/mL. The impedance magnitude spectra of EBV samples did not change with respect to the control samples, however the impedance magnitude of HIV and mixture of HIV and EBV samples decreased compared to the control samples.*

To the best of our knowledge, there is no accurate, inexpensive detection method to diagnose HIV during the acute stage at the POC. This rapid, simple, inexpensive, and label-free electrical sensing method is the first demonstration of viral detection utilizing viral nano-lysate at virus concentrations that occur at the acute stage of HIV-1 infection. Utilizing pathogen nano-lysate's electrical fingerprint in an electronic read-out as a powerful and sensitive detection signal may open exciting avenues in creating practical POC diagnostic tools for infectious diseases. This label-free electrical sensing method of pathogen detection using impedance spectroscopy of pathogen lysate constitutes a broadly applicable platform for detection of other viral and bacterial pathogens in infectious diseases with reasonably well-described biomarkers including herpes, influenza, hepatitis, pox, malaria, and tuberculosis.

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