A low-cost diagnostic technology for HIV detection

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Abstract

Introduction: A new detection model directed at the coordination and simplification of global HIV treatment is described in this paper. It provides both health workers and patients worldwide with important medical information regarding disease status in real time, at home. We developed an innovative monitoring technology that significantly streamlines HIV treatment strategies and thus leads to more effective control of HIV infection.
Materials and Methods: This technology is built around an original bionanosensor (BNS) detection device, which utilizes piezoelectric technology to detect and track HIV infection within hours of initial exposure to this virus. The BNS device rapidly, and specifically, detects even low levels of HIV-1 p24 core protein within a small sample.
Results: BNS assays were conducted with small volume samples (10–100 µl) in a 10–20 mm^3 detection chamber, and results were obtained within 5–15 minutes of test initiation. This assay was able to detect of 0.3–3.0 ng/ml of p24 protein, which was validated with a parallel ELISA analysis. These levels are typically observed during the early onset of infection and/or in individuals undergoing HAART therapy.
Conclusions: The BNS assay will provide a specific and personal health profile or health signature of HIV/AIDS patients. The potential applicability of the BNS for use in multiple clinical and point-of-care settings worldwide is considerable. It lowers the cost of HIV detection and treatment as well as significantly improves patients' quality of live. Finally, this technology will provide a platform for the detection and treatment of numerous other viral infections and health conditions.

Key words: HIV-1, p24, bionanosensors, HIV/p24 blood detection.

Introduction

Nearly 38 million individuals currently live with HIV. Effective prevention measures and treatment regimens are limited in large degree by the lack of a rapid, sensitive and low-cost means to directly measure this virus. The rapid detection of HIV following exposure, and monitoring of HIV levels in infected individuals, are critical components of the worldwide HIV infection control strategy. While humanitarian efforts have increased both HIV/AIDS awareness and prevention, and the number of infected individuals on low-cost highly active anti-retroviral therapy (HAART) continues to rise, there remain nearly two million newly diagnosed infections each year [1].

The benefits of rapid testing to determine the status of HIV infection are well established. The introduction of HAART treatment within hours of exposure has been shown to dramatically limit both virus spread and progression to AIDS [2]. Equally critical is the need to continuously monitor virus load in individuals on HAART and/or other HIV therapies (despite their toxicity and adverse reactions) as well as those participating in emerging vaccine trials. With the expansion of HAART in countries with poor access and/or quality health care, individual variations in HAART effectiveness are rarely monitored. Unfortunately, these individuals may reach, with tragic consequences, the mistaken assumption that they are either virus-free or incapable of transmitting HIV [1, 3, 4].

Diagnostic tests for HIV based on the presence of antibodies are relatively rapid and low cost, but these tests are not suitable for monitoring chronic active infections, as antibodies to HIV are constitutively present at high levels. Similarly, these tests are not appropriate for assessing infection in individuals who may have received an HIV vaccine. Lastly, as antibody levels rise slowly after virus exposure, these kits may not reliably measure HIV infection until at least 3–4 weeks after the initial contact. Current approaches to measure virus rely predominantly on laboratory-based tests that require access to a health clinic and trained technical staff. For example, DNA/RNA-based PCR tests can effectively monitor infection levels but require thermocyclers, multiple reagents and routine laboratory-based standardization. Similarly, p24 ELISA/SPR analysis can also be used to confirm infection and viral replication but it must also be performed in a laboratory with dedicated equipment and trained personnel. The lack of ready access and/or the associated costs of such assays prohibit continuous monitoring of HIV levels for most individuals in low- and middle-income countries, which often face the burden of persistent HIV infection in the population. As a result, even patients with access to HAART therapy are often monitored infrequently, and many individuals on HAART in rural or remote settings are simply not monitored at all [5–8].

In this paper we describe a portable, disposable and inexpensive multiresonant thickness shear mode (TSM)-based bionanosensor (BNS) device for the early detection and treatment monitoring of HIV. Piezoelectric high frequency BNS technology offers an attractive approach to measure chemical and biological elements at the nanoscale, are inexpensive to manufacture, and are micro-scale [9–11]. The device described herein detected the major HIV-1 core protein p24 at the ng/ml scale in plasma and holds the potential for development of a revolutionary, low-cost, hand-held device that can be utilized in point-of-care settings without the need for expensive equipment or technical staff. This device was used, with great success, to detect gp120 virus protein and the BNS technology was previously described and discussed in detail [12].

Materials and Methods

Device hardware

The key element of the BNS is the thickness shear mode (TSM) sensing microstructure. The important working parameters include the operational frequency, the dynamic range, and the noise level. The operational frequency of the TSM is dependent on the membrane thickness of the sensor. The dynamic range and the noise level are determined by the Q-factor of the TSM, which in turn is affected by the roughness, flatness, and low level of defects in the membrane. As a consequence, well-controlled quartz fabrication processes must be applied in order to meet these requirements. The sensors were fabricated using a dedicated integrated circuit (IC) microfabrication process [13]. Piezoelectric materials (quartz) were cut and polished to the required thickness and shape. The mask for the given electrode pattern was developed and the metal electrodes were either RF sputtered or made photolithographically. High frequency sensors, above 50 MHz, were made using an additional combination of reactive ion (RIE) and chemical wet etching techniques. Electrical connections were made using ultrasonic bonders.

The electronic measurement system

The laboratory measurement system was based on a technique utilizing a network analyzer and a personal computer for data acquisition and signal processing. The principle of operation employed by this technique involves the measurement of the trans-impedance of the TSM sensors. The network analyzer-based method provides a versatile measurement system which allows for rapid and wide frequency band scanning of the trans-impedance characteristics of the TSM sensor. The time and frequency domain signatures of the TSM response to antibody-antigen interactions and the time characteristics (kinetics) are readily obtained. The TSM sensors were measured as a one or two-port device depending on the specific biological measurement requirements. All sensors with their enclosures (the chamber, reference liquid, cables, etc.) were calibrated in order to eliminate the influence of ambient conditions on the results. The measured sensor parameters that were used for data processing and subsequent biological interpretation included the sensor resonant frequency, magnitude, phase, impedance,

and their signatures in the time domain. For the sensor array a system of electronically controlled microwave switches was used to change between different TSM sensors.

Bionanosensor device preparation

To establish proof-of-principle for the efficacy of the BNS sensor in detection of HIV we used the well-established HIV biomarker p24, as the concentration of this protein in blood correlates directly with the level of virus infection. As described below, the presence of virus protein was determined by attaching anti-p24 antibodies to the TSM sensor surface, then, after washing, test samples were exposed to the BNS interface, and the level of protein binding was measured by changes in the resonant frequency of the BNS sensor. Establishing the correct antibody immobilization procedure to capture the biomarker of choice is critical to sensor performance. The gold electrode surface of sensors was cleaned using Piranha solution (one part 30% H_2O_2 in three parts H_2SO_4) [14]. After 2 min exposure the sensor was rinsed with distilled water and the surface was dried in a stream of nitrogen gas. Prior to utilization, the sensor was immersed in phosphate buffered saline (PBS). To coat the sensor surface with binding reagent, an aliquot (5 ml) of the capture antibody (anti-p24, PerkinElmer, Boston, MA) was introduced to the sensor surface at 5/mg/ml, incubated for 60 min, and then washed free in PBS.

Performance testing and optimization

We measured the electrical response of the TSM sensor by calculating the change in the frequency as a function of time for the electronic detection system in the vicinity of its operating frequency range. The magnitude of the response, the S21 scattering parameter, is defined as $|S21| = 20 \log (100/(100 + Zt))$, and Zt = total electromechanical impedance of the TSM sensor that is a function of the liquid loading. When the BNS is loaded with biological media, the sensor response S21 will exhibit a shift in resonant frequency and a decrease in magnitude. These changes can be correlated with mass accumulation on the sensor interface because of the binding between antibody and antigens. Depending on the antibody-antigen interactions at the sensor surface-medium interface, a positive or negative shift can be seen in the frequency response [15].

Analysis of anti-p24 BNS performance characteristics was first conducted using an isolated preparation of p24 (Pierce, Thermo Fisher Scientific., Rockford, IL) at 1 pg–100 ng. Subsequent to this, samples of plasma and serum from HIV-1 positive and negative individuals were also examined. These samples were obtained from the University of Pennsylvania Center for AIDS Research core facility. The level of virus and p24 in these samples was determined by RT-PCR, using the bioMerieux NucliSens EasyQ HIV-1v1.1 assay (bioMerieux, Inc, Durham, NC), and by p24 ELISA as described below.

P24 ELISA assay

To confirm infection and viral replication, p24 ELISA was used. P24 ELISA reactions were performed using an Alliance HIV-1 p-24 ELISA kit (PerkinElmer, Boston, MA). Briefly, serial dilutions of human plasma or serum were placed in a 96-well ELISA plate with TritonX-100 and incubated for two hours at 37°C. Following intensive washing and addition of the detector antibody, the plate was incubated for one hour again at 37°C. Subsequently, the experimental samples were incubated with Streptavidin-HRP for 30 minutes at room temperature, treated with OPD Substrate Solution and read at 492 nm in the Plate Reader MRX Revelation (Dynex, Chantilly, VA). The final viral concentrations were evaluated on a Macintosh computer OS X Snow Leopard Version 10.6.8 using Microsoft Excel 2011.

The acquisition and use of human samples were reviewed and approved by the University of Pennsylvania Institutional Review Board (IRB) – protocols: #803567 and #809496.

Results

Device configuration

The device consists of an independent chamber housing the TSM sensor encased in a disposable biochip. The sensors are connected to a piezoelectric transducer and excitation elements as well as electrodes and micro-circuitry for monitoring and reporting TSM modulation upon sample binding. As noted in Materials and Methods, the sensor is coated with an anti-p24 antibody and the reaction is initiated by the application of a 10–100 µl sample. The readout data provide precise information on HIV infection by transmitting p24 concentration/ml. The kinetics of the detection process and the time of the response by the sensor are termed the signature. The signature can be used to set sensor thresholds that return only data generated within the operating envelope of the sensor, significantly diminishing signal noise and improving reliability. The combination of these features creates a smart, intelligent sensor (Figure 1).

HIV sensor testing

Initial device testing and calibration was performed using a monoclonal mouse anti-HIV-1

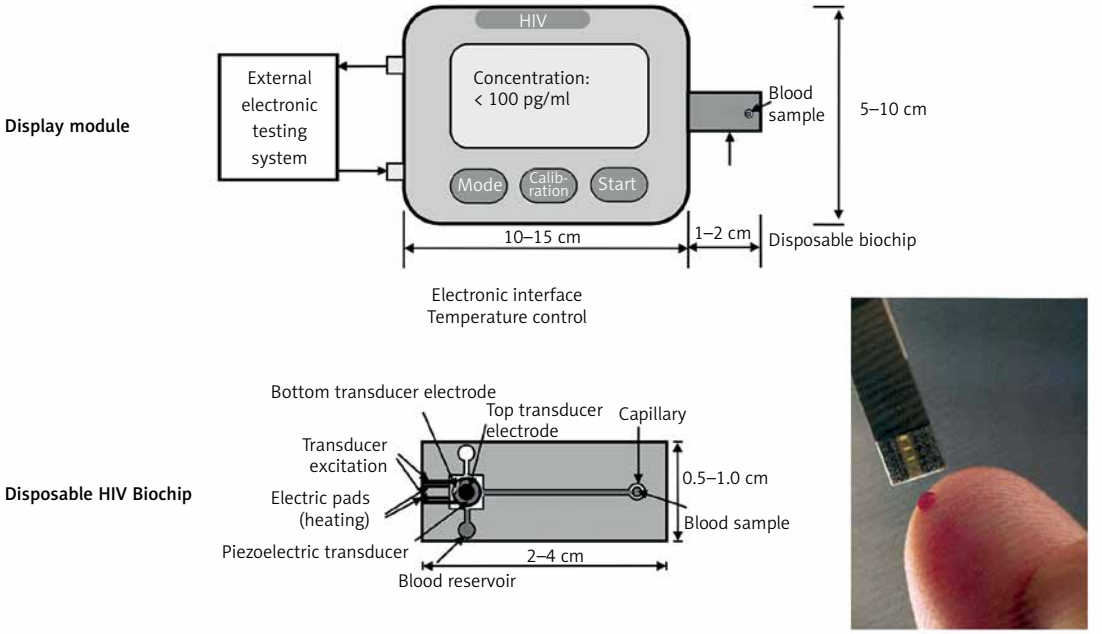

Figure 1. Display module of HIV Biochip cross-section (top view)

p24 antibody and a commercial preparation of p24. The TSM sensors were placed in a custom fabricator holder and saturated with antibodies to p24 protein as described in Methods. After extensive washing, the sensors were probed with either BSA or purified p24 protein (1 ng/ml), and binding to the sensor was observed at fundamental frequencies (15–100 MHz). A computer was used to control and collect data from a network analyzer (HP4395A), which drives the sensor and monitors the sensor response. As shown in Figure 2, the binding of p24 resulted in a marked frequency change (negative deflection) relative to the BSA protein control.

In a subsequent experiment, the fabricated 100 MHz fundamental frequency chip was enclosed in a protective PDMS-PMMA hybrid cartridge that allowed it to be connected to a laboratory-based measurement system (network analyzer and radio frequency switch) and a multichannel peristaltic pump. As above, sensors were coated with anti-p24 antibody, washed and then blocked with BSA prior to incubation with p24 HIV-1 protein in a maximal sample volume of 100 µl. Real time detection of p24 at 0.3–3 ng/ml was then recorded over 15 minutes, the results of which are shown in Figure 3. A linear plot of p24 detection sensitivity, shown in Figure 4, demonstrates that p24 binding is linear over the range of 0.3–3 ng/ml.

Bionanosensor detection of HIV in human plasma

With knowledge of both BNS binding specificity and sensitivity to p24 in hand, analysis was extended to the direct detection of HIV-1 p24 in plasma samples obtained from 20 individuals infected with HIV-1. The p24 concentration of these samples ranged between 1.20 and 11.08 ng/ml as determined by ELISA. So that these experiments

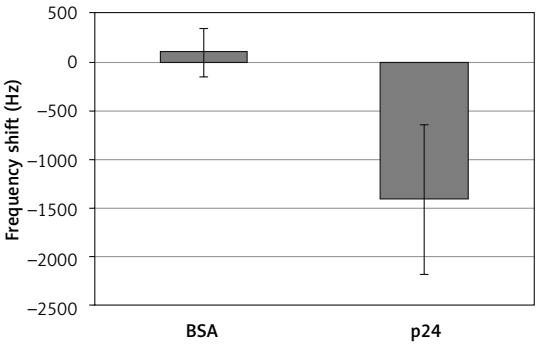

Figure 2. Antigen specificity. Binding of p24 versus BSA to sensor-anti-p24 interface. In this system a positive signal generates negative inflection

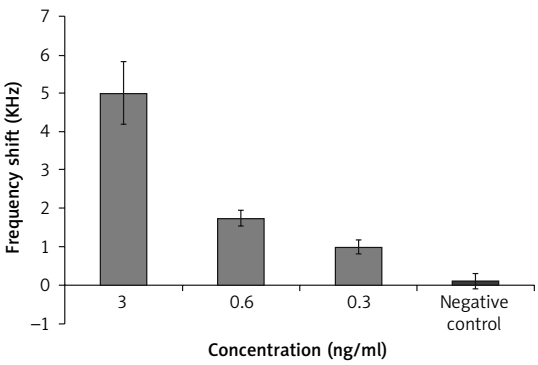

Figure 3. Frequency shift as a function of p24 concentration. Negative control – 5% BSA

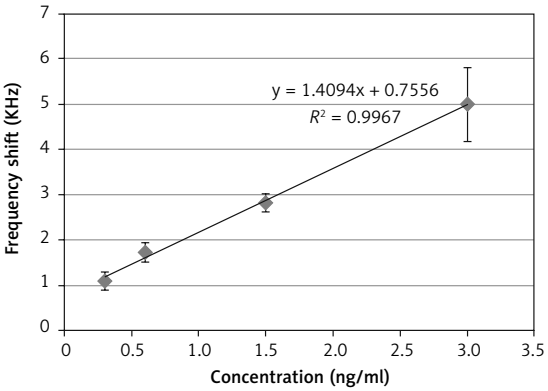

Figure 4. p24 detection sensitivity curve

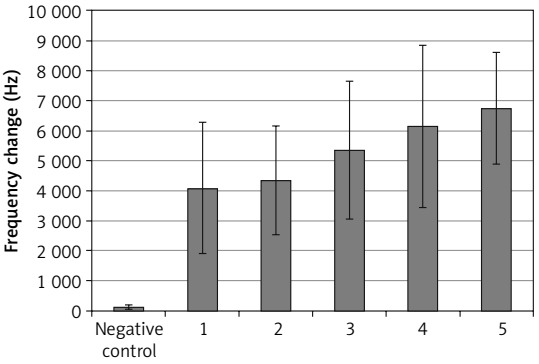

Figure 5. Bionanosensor analysis of HIV detection in human plasma samples (1–5)

might be conducted without unnecessary health risks, all samples were first rendered replication defective by heat treatment. Plasma aliquots were first analyzed using sensors that were not coated with anti-p24 antibody to establish the baseline response. The BNS frequency change in 100 µl samples from HIV-1 seropositive individuals as contrasted to a seronegative control was then measured on sensors coated with anti-p24. Results shown in Figure 5 display the most prominent example of the BNS sensor response relative to virus/p24 concentration. The results demonstrate a clear correlation between BNS signal intensity and HIV-1 p24 concentration in human plasma samples

Discussion

The results of our study demonstrate the feasibility of BNS technology for the specific detection of HIV protein in blood plasma. Importantly, BNS assays were conducted with small volume samples (10-100 µl) in a 10–20 mm^3 detection chamber, and results were obtained within 5–15 minutes of test initiation. Assay sensitivity was linear over the range of 0.3–3.0 ng/ml, and mirror responses were observed with parallel ELISA analysis, which supports the utility of this approach for measuring meaningful levels of virus in early periods of infection and/or of individuals under HAART therapy. These results also point to the broader utility of the BNS in detecting virus in other body fluids and other blood metabolites for which antibodies or other binding reagents exist.

Recent progress in micro-electronic and micro-mechanical fabrication technologies offers exciting possibilities for the development of a new class of devices that measure chemical and biological elements at the nanoscale [9–11]. Piezoelectric high frequency BNS provide an attractive platform for such sensors as they display high sensitivity and specificity, small size and portability coupled with a fast response time. Sensors based on this technology can be produced using standard photolithography and have excellent aging characteristics. As solid-state devices they can also be synthesized as disk, plate or prism shapes and manufactured with metal electrodes to enable a seamless interface with various electronic circuits (IC, MEMS and NEMS), thus facilitating user-friendly read-outs [16–19].

The key element of the BNS assay is the TSM sensor, which possesses the unique property whereby an applied electrical voltage induces a shear mechanical strain, over a broad frequency range. By exciting a sensor with an alternating voltage, standing acoustic waves are produced within the sensor and the sensor behaves as a highly sensitive electromechanical resonator, transmitting a shear wave into the liquid medium. The shear wave penetrates liquid over a very short distance, in the order of tens to hundreds of nanometers, and the influence of the interfacial conditions on the behavior of the sensor is very strong. The shear acoustic wave decays rapidly with a rate determined by the penetration depth factor, which is proportional to liquid viscosity and inversely proportional to liquid density and the frequency of the wave. Therefore, by changing the frequency one can control the distance at which the wave probes the sensor-liquid interface. Multiresonant operation of the BNS as applied in our system allows one to control the depth from which each sensor response is being collected, effectively slicing the medium, which substantially improves the sensor performance in terms of sensitivity, selectivity and resolution.

In addition to the detection of gases and viscoelastic properties of liquids [20–26], a number of piezoelectric biosensors have been developed for medical applications. Similar to our approach, the most common configuration utilizes an immuno-biosensor to which antibodies or antigens are immobilized on the sensor surface [27]. Current applications include measurement of antibodies specific to herpes virus in plasma, detection of human cells, herbicides in drinking water, *Staphylo-*

coccus aureus bacteria, drugs and toxins in human urine, and viruses such as hepatitis and African swine fever [28–34].

In summary, our results demonstrate the utility of a bionanosensor for the detection of HIV-1 protein in human plasma. The primary advantages of this system are sensitivity, specificity, electronic integration and production cost. While there are several other excellent means to measure the concentration of HIV-1 in body fluids, our approach offers the specific advantage of developing a small portable device to monitor individuals afflicted with HIV who are in remote settings without ready access to well-equipped health clinics. This technology also holds promise for expanding the diagnostic capacity of BNS by increasing the range of molecules detected, such as gp120, and CD4 and CD8 counts, either in parallel or in sequence on a single device. This also means that this technique may be used in the future for detection of a variety of different infections and co-infections as well.

In conclusion, we describe here a BNS device that is sensitive, rapid, portable and inexpensive when produced on a commercial scale, that is capable of detecting the presence of p24 HIV-1 protein at ng/ml levels in less than 15 minutes while using no more than 10–100 μl of blood.

Conflict of interest

The authors declare no conflict of interest.

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