Project Description

Abstract

Human Immunodeficiency Virus (HIV) is a major global health issue. Approximately 35 million people worldwide are living with HIV, a majority of whom live in resource-limited settings. The World Health Organization recommends that every person diagnosed with HIV receives a viral load test at least once per year for staging, treatment decisions, and monitoring of treatment efficacy. The standard laboratory-based viral load test requires advanced infrastructure, a well-controlled environment, and trained technicians. These tests are not applicable for resource-limited settings. As such, there is a need for the development of inexpensive, portable, and easy-to-operate technologies for HIV treatment monitoring that can be used at the point of care. Point-of-care viral load testing has improved over the past few years, and there are multiple new technologies in development. However, there is no "silver bullet" technology that has the potential to change the landscape of viral load testing in development yet.[1] We present a microfluidic solution for the capture and quantification of HIV. We have transformed a novel porous membrane that has been proven effective in capturing HIV into a system that is capable of moving from sample to answer on-chip. In doing so, we have created a technology that is appropriate for point-of-care applications. Our innovation includes the incorporation of a cyclic voltammetric system into microfluidic devices containing a porous membrane. This system is able to quantify viral loads of 1,000 copies per mL, which is the limit of detection required for point-of-care viral load technologies as recommended by the World Health Organization. In addition to technical feasibility, our design compares favorably to other technologies in development in terms of per-test cost and is more advantageous in terms of time to obtain results, simplicity of use and projected overall instrumentation cost. These improvements over current technologies indicate that our design could be the "silver bullet" technology that has the potential to dramatically increase access to viral load testing globally.

Clinical Need

Currently, 35 million people in the world are living with HIV, the majority of whom reside in low- and middle-income countries.[2] To diagnose, stage and monitor HIV infection and progression, viral load measurement is an imperative test. The World Health Organization (WHO) recommends viral load tests at least once per year for every person who begins antiretroviral therapy (ART) to stop the progression of an HIV infection.[3] Conventionally, viral loads are measured using central laboratory-based tests, which require infrastructure, cold-chain transport, and trained personnel. To address the global pandemic of HIV, tests designed for use at the point of care that can be run with a portable setup, with a turn-around time of less than an hour, and require minimal training are urgently needed.[4]

Viral load tests are preferred for treatment monitoring because of their ability to detect virological failure earlier than immunological response or clinical symptoms and to indicate the need for adherence support or a change in treatment regimen.[6] Most effort is focused on miniaturizing nucleic acid amplification-based tests, and significant progress has been made in the past decade in simplifying the amplification and detection schemes.[7] Yet, the equipment cost is still prohibitively high for clinics in low- and middle-income countries, generally upwards of $10,000 (USD), and the operation complexity requires trained technicians.[3]

Design

We present an alternative approach to HIV viral load testing with the potential to be significantly easier to operate, have a much faster turnaround time, and be less expensive than

nucleic acid amplification-based tests. This lab-on-a-chip design utilizes microfluidics for capture and quantification of whole HIV virions. The small size of microfluidic devices and the potential to automate assays make microfluidic technology appealing for point-of-care settings. Directly detecting whole particle virions instead of their molecular fingerprints, minimizes sample preparation procedure, resulting in a faster sample-to-answer timeframe.

To isolate virions from a solution, such as plasma, we are using an immunoaffinity microfluidic channel. Traditional flat-bed microfluidic devices lack capture efficiency because viruses are much smaller than the channels within the devices. The Cheng laboratory from Lehigh University has demonstrated the ability of a porous membrane incorporated into a microfluidic device to isolate approximately 80% of HIV virus from a solution compared with approximately 10% capture using a flat-bed device.[8] Figure 1 shows the steps of fabricating the porous membrane, including depositing of a binary suspension of silica and polystyrene beads (Figure 2A), melting polystyrene, and etching out silica, leaving behind a porous structure (Figure 2B). The overall process is illustrated in Figure 2. The pores have regular shape and sizes comparable to HIV virions. However, in order for this novel technology to be applicable for use at the point of care, a quantitative analysis system allowing for on-chip detection of captured virions needed to be incorporated into the devices.

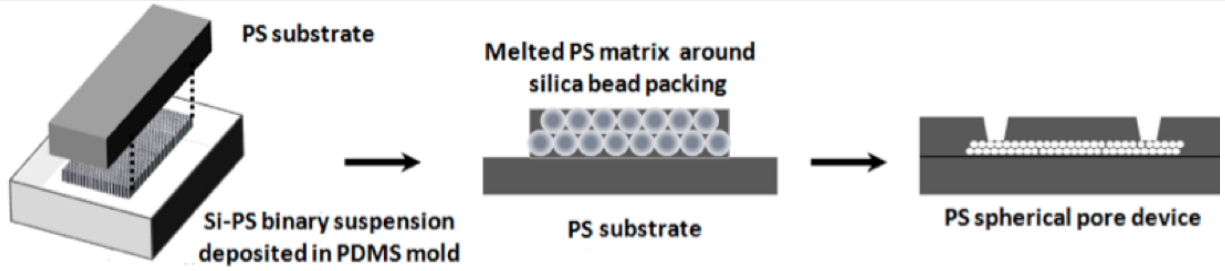

Figure 1: Method of fabricating porous microfluidic membrane. A binary suspension of polystyrene and silica beads is deposited into a PDMS mold, polystyrene is melted, and silica is etched, leaving behind a porous membrane. *Source: Surawathanawises et al.*

Our team addressed the challenge of transforming the porous membrane into a technology that can go from sample to answer, a necessary innovation to allow for the use of this design at the point of care. To do so, we incorporated electrodes into the microfluidic devices containing porous membranes for electrochemical detection of captured virions. Cyclic voltammetric analysis has been validated for biomarker detection within microfluidic devices with internal geometric features, and the sensitivity and quantitative characteristic are suitable for viral load tests.[9] To perform cyclic voltammetry, we first inject a redox solution containing free ions into our device. When voltage is applied cyclically to one electrode (from -0.3 V to +0.4 V), ions move through the membrane to the other electrode. If no virus is present in the membrane, ions are able to move freely through the membrane and reach the electrodes, and the resultant peak current value is high. However, when there are blockages within the membrane due to captured virus, which prevent ions from reaching the electrode, peak current values are lower.

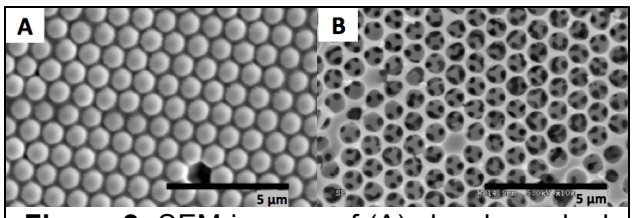

Figure 2: SEM images of (A) closely packed silica beads with polystyrene beads filling interstitial voids corresponding to the first step of Figure 1, and (B) resultant porous membrane corresponding to the third step of Figure 1. *Source: Surawathanawises et al.*

To create blockages, we build aggregates around captured HIV (Figure 3). More specifically, the membrane is functionalized with an HIV-specific antibody, anti-gp120. Next, HIV is captured by this antibody. To enhance blockage, gold nanoparticles are bound to the captured HIV, and finally, silver is deposited around the gold nanoparticles. Now, when redox solution is injected, and voltage is applied, ion movement is blocked by the aggregates, and the resultant peak current reading is lower. This inverse relationship allows for the measurement of peak current values in order to diagnose a patient's viral load. An example of cyclic voltammetric curves for two viral loads (0 copies/mL and 1,000 copies/mL) with peak currents highlighted in red is shown in Figure 4.

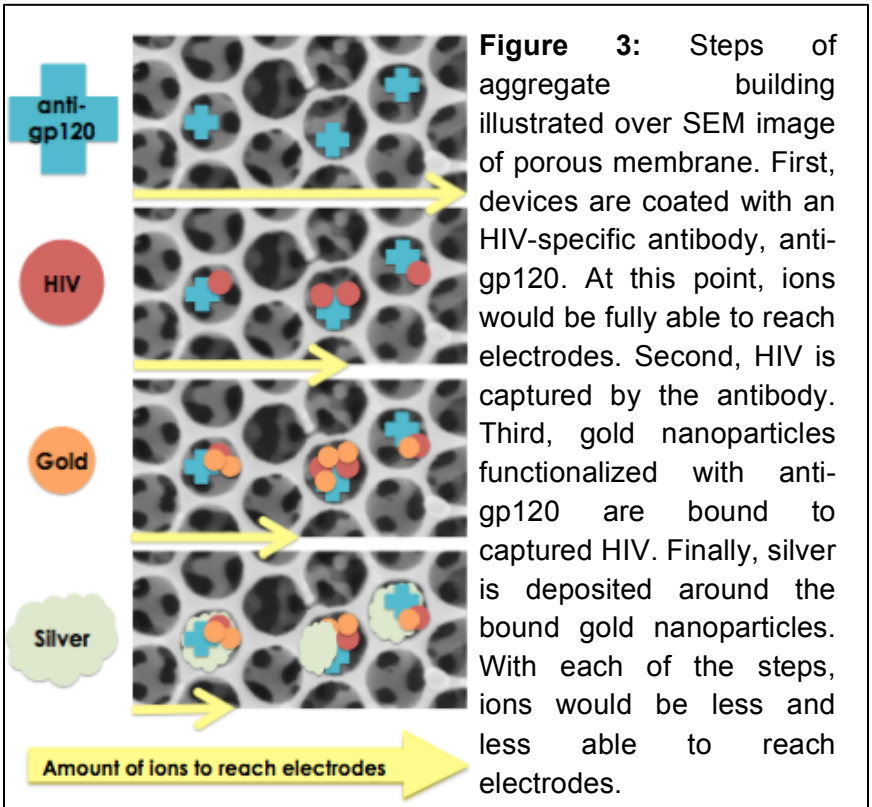

Figure 3: Steps of aggregate building illustrated over SEM image of porous membrane. First, devices are coated with an HIV-specific antibody, anti-gp120. At this point, ions would be fully able to reach electrodes. Second, HIV is captured by the antibody. Third, gold nanoparticles functionalized with anti-gp120 are bound to captured HIV. Finally, silver is deposited around the bound gold nanoparticles. With each of the steps, ions would be less and less able to reach electrodes.

The innovation presented here is the application of cyclic voltammetry into a novel microfluidic membrane geometry optimized for HIV capture. Dr. Krissada Surawathanawises worked with Kathryn Kundrod, a member of the student design team, to develop the porous capture membrane with uniform pore structure and high porosity. Kathryn also assisted with testing of viral capture in the porous membrane. The student team then researched different methods for nanoparticle and biomolecule quantification and adapted cyclic voltammetric methods to the viral capture device. In order for these methods to be applied to the porous microfluidic device, the team redesigned the device by incorporating electrodes. Further, the student team developed a more efficient device fabrication process through the use of water jet cutting of substrates and electrodes. The final device design is shown in Figure 5. All experimentation to implement and test cyclic voltammetry was designed and optimized by the student team under the guidance of Professors Xuanhong Cheng, Steven McIntosh, and Yevgeny Berdichevsky of Lehigh University. While most of the capture

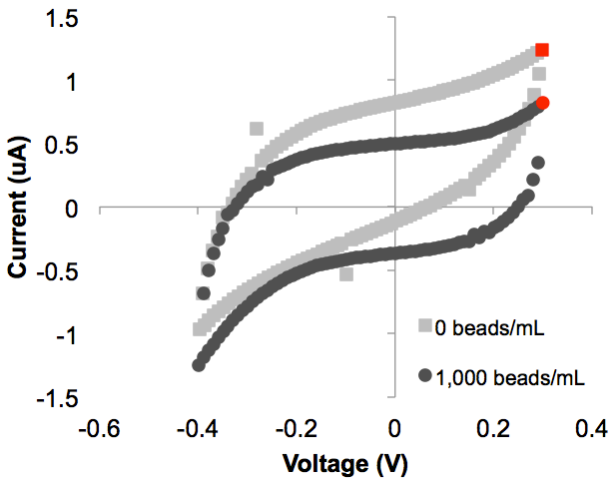

Figure 4: Cyclic voltammetric curves for two viral loads. Peak current is highlighted in red on each curve.

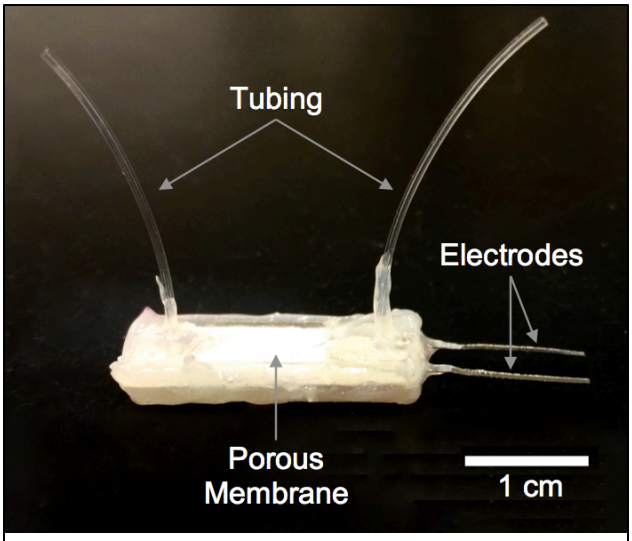

Figure 5: Final microfluidic device design with electrodes incorporated for cyclic voltammetry.

experiments to date were performed using synthetic nanoparticles, the team has won a Lehigh University Undergraduate Grant to evaluate the device performance using lab stock of HIV virus in the summer of 2015.

Evidence of Working Prototype

Technical Feasibility

Due to biosafety considerations, experimentation was first carried out with simulated HIV (biotinylated polystyrene beads 100 nm in diameter). To simulate the capture reaction, devices were coated with neutravidin, which has a strong binding affinity to biotin. To test the extent to which peak current and viral load have an inverse relationship, four concentrations of simulated virus (0, 1,000, 10,000, and 100,000 copies/mL) were flowed through devices, and peak currents obtained during cyclic voltammetry were recorded. The relationship proved linear with a fairly strong correlation (R^2=0.93357), as can be seen in Figure 6.

The World Health Organization defines the threshold of virological failure as a viral load of 1,000 copies/mL. Therefore, for point-of-care viral load devices to be clinically relevant, they must have a limit of detection below or equal to 1,000 copies/mL. Our device was able to differentiate between a control viral load of zero copies/mL and the threshold viral load of 1,000 copies/mL, indicating clinical relevance of our design (Figure 7).

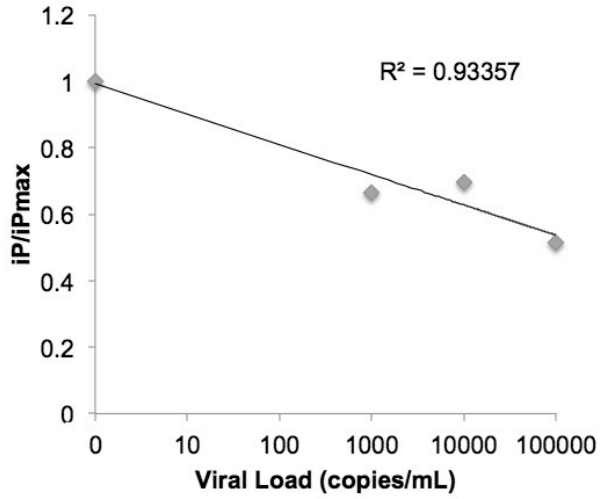

Figure 6: Standardized peak current versus viral load. Relationship between peak current and viral load is inverse with a linear correlation as expected. This relationship will be used as a calibration curve to calculate viral load in an unknown sample through cyclic voltammetry measurement.

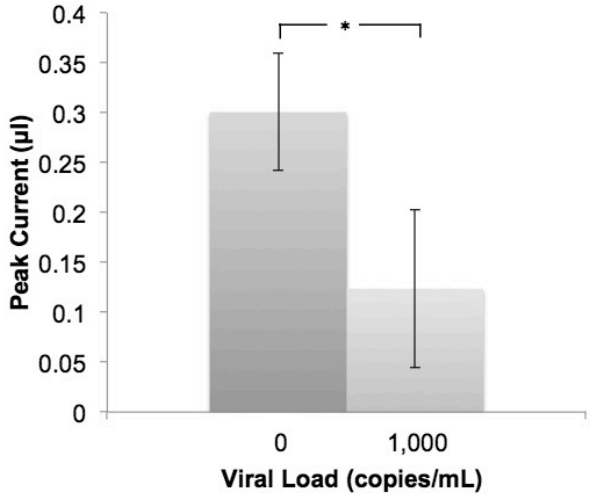

Figure 7: Differentiation between control viral load (0 copies/mL) and threshold viral load for adjusting treatment regimens (1,000 copies/mL). Asterisk indicates a statistically significant difference (p=0.0244) between peak currents measured for each viral load. Therefore, limit of detection is appropriate for point-of-care applications.

Potential for Use at the Point of Care

Finally, we optimized the flow rates in our assay in order to minimize total testing time. We found an optimal flow rate for each step of aggregate building to be 15 uL/min. Using a sample size of 100 uL for each reagent, total testing time could be as low as 29 minutes, which is the time necessary to run the assay. Additional time might be introduced with steps such as loading samples and switching reagents, though these steps are only anticipated to increase total testing time by a few minutes. Optimization of these steps will happen in the next phase of development. Keeping testing time under an hour makes this device particularly suitable for point-of-care applications.

Reagent cost is projected to be $2.38 per device (Table 1), which compares favorably to other point-of-care viral load technologies in development.[10] Additionally, operation of our device is simpler, and our instrument cost is projected to be significantly lower, both of which make scale-up of manufacturing and distribution more feasible.

Future Work

Moving forward, Cyclic Solutions plans to continue testing this device to generate a more robust standard curve relating viral load to peak current values. The team has a proposed timeline of two months of testing using HIV pseudovirus. Following testing, Cyclic Solutions intends to design instrumentation for the use of this device at the point of care. The instrument will need to include a low-cost potentiostat along with a microcontroller. The team anticipates the total cost of instrumentation to be on the order of $1,000 in comparison with costs on the order of $10,000 for instrumentation required for nucleic acid amplification tests.[1] Further, it will need to include a mechanism for switching between the reagents that will be used in testing.

Cyclic Solutions is currently working with the Baker Institute at Lehigh University to secure intellectual property rights through a provisional patent. The team will look to transfer these rights to a diagnostic device company capable of manufacturing the device on a larger scale in order to reduce per-test cost. The team will also ensure that the manufacturer complies with ISO 13485:2003, the internationally recognized standard for quality assurance of medical devices.

In the next few years, Cyclic Solutions would like to see its design, Viral Diagnostic Technology, obtain procurement from a UN organization such as the WHO or UNITAID. With

Table 1: Bill of materials for reagents and consumables

ID	Part Name	Part Description	Qty/Unit	Build/Buy	Cost per Unit*	Total Cost
1	Microfluidic Chip	Custom printed chip	1	Build	$0.65	$0.65
2	Anti-gp120 Antibody	Ensures only HIV are captured	1	Buy	$0.84	$0.84
3	Gold Nanoparticles	Attaches to virus and allows for silver to deposit	1	Buy	$0.03	$0.03
4	Silver Solution	Deposits on gold to increase size of aggregates	1	Buy	$0.24	$0.24
5	Redox Solution	Provides ions for cyclic voltammetry	1	Buy	$0.01	$0.01
6	Stainless Steel Electrodes	Conducts voltage into device	2	Build	$0.01	$0.01
7	Syringe	Inputs sample into device	4	Buy	$0.15	$0.60
	*Estimates are for production in quantities of 1,000					$2.38

ISO 13485 compliance and procurement from a UN organization, Cyclic Solutions' Viral Diagnostic Technology can apply for the Prequalification of In Vitro Diagnostics Programme through the World Health Organization for facilitated scale-up in an equitable manner.

Cyclic Solutions will develop appropriate data collection and management systems in order to assess the success and iterate its design, if needed. Data collected will be important in order to apply for FDA approval. Cyclic Solutions intends to apply as a Class II medical device through a Premarket Notification 510(k), using aspects of other point-of-care diagnostic tests as predicates.

Potential Impact

With technical and financial improvements over current approaches, our design has the potential to be the revolutionary technology for HIV viral load diagnostics that society currently lacks. The World Health Organization projects that there are over 15 million people who are currently on a treatment regimen. The overwhelming majority of these individuals of are living in low- and middle-income countries,[11] and all of whom are recommended to receive routine viral load tests, which are critical for effective HIV monitoring. A lack of resources keeps people from effectively monitoring treatment regimens. With a design that drastically increases accessibility to viral load testing, Cyclic Solutions' Viral Diagnostic Technology has the potential to help patients and clinicians better manage HIV infections, preventing mortality from failing treatment regimens. As such, our technology will increase life expectancies among people living with HIV, regardless of the resources locally available.

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