Reusable Microfluidic Device for Rapid Detection of HIV

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Team Y4

AJ Senthilkumar, Sean Sullivan, Zach Niswonger, Jess Timog

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SPECIFIC AIMS

Human immunodeficiency virus (HIV) currently has no cure and causes a weakening of the immune system that eventually becomes deadly. Current treatments and testing methods have recently advanced in first world countries to help significantly reduce the danger of HIV, but these advancements are not ubiquitous in countries with poor access to medical resources. Moreover, early detection of HIV is critical in reducing the damage caused by this virus, so the efficiency and speed of testing are of importance. Our long-term goals are to develop a lab-on-a-chip capable of diagnosing HIV in a rapid timeframe with high ease of use and low cost in comparison to other methods that can be deployed in impoverished areas. This chip will help to ensure early diagnosis of HIV and allow infected patients to receive treatment sooner.

Our lab-on-a-chip design synthesizes discoveries made by many previous scientists. Firstly, we utilize the separation of blood into plasma via multiple bifurcation channels to separate blood. Purified blood is semi-transparent, allowing us to mix it with a solution consisting of gold nanoparticles, monoclonal and polyclonal anti-p24, hydrogen peroxide, and streptavidin-catalase conjugate for a visual inspection. Due to the reduction of the gold ions with the hydrogen peroxide, this solution changes color based on relative presence of the p-24 protein. This change in reduction rates also affects aggregation patterns of the gold nanoparticles, which creating the observed color change. We propose the development of our chip will provide ease of use to underserved communities at a lower cost than traditional testing. The following aims lead to the best possible product following this hypothesis.

Aim 1. To construct and test a lab-on-a-chip capable of detecting the presence of the p24 protein via plasmonic ELISA. Current methods of HIV detection require blood samples to be sent to laboratories for extensive testing, including multiple different assays conducted on the sera of the patient. This lab-on-a-chip method would provide greater ease of use and much faster results at a lower cost for patients, which would allow the patient to find prompt treatment should their result be positive.

Aim 2. To optimize the design of the chip, including materials, pumps, design features, and reagents used in detection to minimize cost. As this chip is intended to be used in low-income regions worldwide, it is crucial to minimize the cost of both the chip and its required reagents. Therefore, the concentration and quantity of reagents must be minimized, while still optimizing accuracy.

Aim 3. To determine a method of production that allows large amounts of the chip and required solutions of nanoparticles, p24 antibody, streptavidin-catalase conjugate, and hydrogen peroxide to be created at minimum cost, then distributed to areas in need. We must determine the most effective way to produce our chip design on a large enough scale for widespread use, as well as methods to pre-mix large amounts of the testing reagent. Methods of distribution, target areas, and possible distribution partners will also need to be determined.

BACKGROUND

There are two types of HIV: HIV-1 and HIV-2. HIV-1 is most commonly found and HIV-2 is less infectious. For our lab-on-a-chip, it is detecting HIV-1 and it is crucial to this disease for rapid identification due to the devastating impact it can have on the immune system. HIV can be easily transmitted through the contact of an infected person's bodily fluid, entering the bloodstream of a healthy patient via a mucous membrane. Examples of transmission would be through unprotected vaginal or anal sex, sharing of needles, childbirth of a HIV-positive mother, or being bitten by an HIV-positive patient. There are three stages of HIV: Acute, Chronic, Acquired Immunodeficiency Syndrome (AIDS). Once a person is infected, they begin presenting flu like symptoms 2 to 4 weeks afterward and classified as having Acute HIV. This disease attacks human T-cells, inhibiting their adaptive immunity and capacity to respond to specific pathogens. The virus also infiltrates T-cells and hijacks their reproduction mechanisms, forcing them to manufacture new viral copies and enabling further spread of HIV virions. If left undetected, the patient's T-cell count drops until it reaches dangerously low levels. This process of infiltration and depletion of T-cells can be seen in Figure 1. As the patient progresses untreated, they classify as Chronic HIV where they are asymptomatic since the virus does not multiply as quickly but can transmit it to others and can last for years until advancing to AIDS. Patients with T-cell counts of <200 have AIDS, a stage that leaves the immune system so depleted that even a minor cold can kill a patient. The progression of this disease can be prevented by early testing if the patient suspects possible exposure. If detected soon enough, a patient can undergo antiretroviral therapy, taking medication to prevent multiplication of the virus and to maintain immunity levels. Depending on the severity, the patient can usually live comfortably, staying on antiretroviral therapy for the rest of their life.

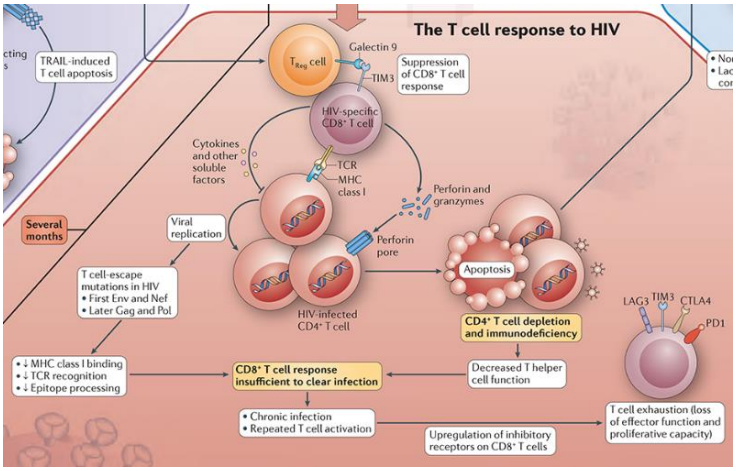

Figure 1: Immune System Reaction to HIV (STEMCELL Technologies)

Currently, there are several modes of detection out in the market. Antibody tests are available, allowing a blood or saliva sample to be analyzed for HIV antibodies. Fourth-generation tests are also popular as they analyze direct HIV virus and antibody p-24 count in human blood. Finally, a Nucleic Acid Test can be done to analyze the quantity of the virus in human blood. While rapid in-home tests can be done and take 20 minutes to display results, they

are rarely accurate and require a healthcare provider's laboratory testing. With an antibody test, the patient must have been infected for at least twelve weeks, and the test itself takes another three days to receive the results. An example of an antibody and antigen test is the Enzyme-Linked Immunosorbent Assay (ELIZA). This is where a blood sample is collected from the patient and sent to a lab where the sample is added to the mixture of HIV antigen and anti-HIV antibodies. The sample and mixture are combined by a machine and if binding is observed, the test results are positive but a secondary test, Western Blot test, must occur to confirm. A variation of this test, differentiation assay, requires the handling of a scientist and specific antibodies and antigens are carefully identified through the binding. False positives can occur due to preexisting health conditions such as lupus or syphilis, thus the need for a secondary test. A Western Blot Test is the secondary test where it separates the HIV antibodies from a blood sample, not just the presence of the virus itself.

This chip utilizes multiple existing technologies. Firstly, it uses a vacuum driven peristaltic micropump with valved actuation chambers. This pumps liquid in a periodic manner, similar to the way a heart pumps blood through the body. It is made using PDMS, which is a soft, malleable plastic. It has 3 chambers, each shut by a valve. When a negative pressure waveform is sent through these chambers, the valves release, allowing fluid to flow through into the next chamber or out of the pump. Then, a positive pressure waveform is sent through the pump, closing the valves and stopping flow. This cycle continues, allowing for highly controllable flow. This device has been used in many microfluidic applications, and its ease of use, lack of internal mechanics required on the chip, and controllability made it the perfect choice of pump. The specific pump being used was created by Cui, J. and Pan, T., and can be found in their article "A vacuum-driven peristaltic micropump with valved actuation chambers."

A second pump is also used in our lab on a chip design, this being the finger actuated pump. This is a pump operated by the user, and is created using PMMA, a malleable plastic. The user simply pushes on the fluid, and the increase in pressure due to this compression forces the fluid to flow. The specific finger pump that will be used in this design can be found in the article "A Controllable and Integrated Pump-enabled Microfluidic Chip and Its Application in Droplets Generating" by Zhao, B., Cui, X., Ren, W. *et al.*

Another existing technology this lab on a chip utilizes is bifurcation channels, which are used to filter liquids, in this case it filters blood to create plasma. It does this by creating a lower flow rate side channel that intersects the main channel. The red blood cells avoid the channel with the lower flow rate, meaning the red blood cells continue to flow exclusively through the main channel, while the plasma will flow into the side channels. In our chip design, we use these bifurcation channels to lead the plasma to its own chamber, as plasma is required for our testing method, not whole blood. Our design was based on that developed by Yang, S., Undar, A., and Zahn, J.D, and can be found in their article "A microfluidic device for continuous, real time blood plasma separation."

The detection chamber also utilizes components from previous studies. Firstly, the method of detection itself is detailed in "Plasmonic ELISA for the ultrasensitive detection of disease biomarkers with the naked eye" by de la Rica, R., Stevens, M. It involves the use of gold

nanoparticles and various antibodies and enzymes. The reaction of the p24 protein present in HIV with this solution leads to a distinct color change, which is the method of detection. Secondly, it utilizes texture on the bottom of the chamber to ensure adequate mixing. This texturing causes turbulent flow upon the introduction of the sample to the reagents. The specific texture being used was not based on any study, however many studies have backed the use of texture to ensure mixing. With these components combined, our team hypothesizes that our lab-on-a-chip will allow detection of HIV to be faster, cheaper, and more user friendly since it takes away the wait and requirement to go to a healthcare's office for confirmation. This will be done through sampling of blood and results to be read by the color change in the detection chamber.

In this proposal, the significance and innovation of our lab-on-a-chip through the extension research for the need of a more accurate, faster, cheaper, portable detection system of this deadly disease. Next, the methodology of our lab-on-a-chip will be demonstrated by the detailed elaboration of each component of the chip and how it is further improving the process of detection. Finally, the need for this lab-on-a-chip is expressed and its positive impact on impoverish and third world countries that do not have access to laboratories and extensive testing for this highly contagious disease.

SIGNIFICANCE

The prevalence of HIV remains alarmingly high in developing countries. As developed nations are able to adequately test anyone with suspected exposure, they have far fewer issues enabling care for those infected. In fact, most HIV-positive patients undergo a series of antiretroviral medications so effective that they have undetectable levels of the virus in their blood and they are no longer able to infect others. Such success has yet to be seen in developing countries, especially regions such as Sub-Saharan Africa, so a new testing device that maximizes access to care for the underserved is essential to promote the health and wellbeing of humanity.

Current detection methods of HIV have particular difficulty expanding to poorer regions of the world as they are costly, resource-intensive, and time-intensive. The most popular methods (antibody and antigen screening) simply become unfeasible to conduct in nations that do not have established healthcare networks. Large laboratories are needed to house the expensive testing equipment, and tests need to be sent in from the doctor's office to the lab via express delivery. As if the delivery process were not time consuming enough, there is an additional 24-72 hour delay in finalizing the results of the test, at which point HIV infection may worsen. This is particularly troublesome in regions without widespread HIV testing as many do not know they are infected before it is too late, and the time for results to come in is simply too long. In extremely poor countries, tests must be sent out of the area to the nearest lab. Depending on geographic location, this could lead to another delay in results. All of these factors lead to poorer regions of the world having little no access to testing. Not only does this lead to a severely worsened outlook for patients, it leads to a much higher chance of spreading, as many HIV positive individuals are unable to get tested, and do not even know they are sick until they finally become symptomatic.

The proposed lab-on-a-chip eliminates these barriers to effective testing as it allows for fast, reliable testing at a reasonable price. The chip is small and portable, as one can fit in the palm of a doctor's hand. This makes it much easier to distribute these chips across areas with less infrastructure and fewer means of delivery. The devices are also durable, as they are made of a hard PMMA material that allows for multiple uses and comes apart easily for cleaning. This durability makes them much safer to use in more harsh settings, like those it is most likely to be used in. The ease of cleaning ensures its reusability while also removing the need for advanced cleaning equipment, as it simply needs to be placed in a cleaning solution and allowed to rest. The device is efficient, as it utilizes a fraction of the blood sent off to testing laboratories and combines it with small amounts of nanoparticles and antibodies. This makes it easier on pateints, as less blood is drawn, and easier on healthcare providers as fewer resources are needed in order to run the test, making the test cheaper. This decrease in price is extremely important, as it will make health care providers more willing to provide help to these regions that desperately need it and will allow communities to finally be able to afford testing. It also saves time as results come within minutes of initiating a test. This means that patients that suspect they may have been infected can go to their nearest health care provider and be tested, and within the hour be receiving treatment, or going somewhere that can provide them with treatment if their health care center is unable to.

The early and rapid testing of HIV in possible vectors is paramount to their health and wellbeing. If tested early enough, individuals who suspect HIV exposure can begin antiretroviral therapy and may effectively block out the virus before it has a chance to incubate inside them. This means that individuals who get tested as soon as they suspect exposure, usually within one day, gain an invaluable advantage and have a chance at blocking HIV infection so they do not have to deal with it for the rest of their life. For individuals who have passed this critical time period, but still have no symptoms, our test effectively determines their HIV status and can allow them to begin therapy fast enough to attack the virus before it can negatively impact the immune system. If left untreated, HIV quickly destroys the immune system, destroying both T-cells and CD4+ cells that create immune responses to pathogens. Individuals can eventually develop AIDS from the virus, and may soon die from a weakened immune system.

INNOVATION

Our chip design leverages existing technologies into a new device at the micro-scale. No Currently, there is no widely used testing method incorporating assays using plasmonic ELISA, the detection method we have chosen for our device. Moreover, HIV tests currently require one to three test-tubes worth of human blood, while our microchip functions at a fraction of the volume. No widely used HIV test is conducted on site, either, as tests are sent in to specialized labs for additional processing. Our chip can provide fast results on site, allows healthcare workers to test patients on site, and requires very few resources to maintain.

Our chip provides increased convenience to healthcare workers as testing can be conducted on site, as compared to previous methods which rely on sending blood samples from

test sites to labs. Since our chip provides nearly instantaneous results with an easy-to-read color change, less time can be spent preparing blood samples for transport and patients can be tested more rapidly. The design is also extremely easy to use, as it does not require any advanced equipment. The sample must simply be loaded into the device, the user must press the finger pump after filtration, and then observe the color change. There is no advanced technology the user must be able to operate or read, and drawing blood is the only skill required, making more people able to use this device.

The microchip is also relatively easy to clean by soaking in a cleaning agent and prepare for testing as it is constructed of two halves that attach together. After each test, the microchip can be taken apart and washed, then re-loaded with antibodies and gold nanoparticles for the next test. The amount of nanoparticles and antibodies needed per test will be very small as the mixing chamber effectively distributes the purified human blood over all the antibodies.

The reusability of this lab on a chip design gives it a huge advantage over the currently existing rapid testing methods, as being able to use the chip over and over drastically reduces the price associated with testing people, and makes it a feasible option for testing at a large scale level. This functionality can be seen below in Figure 2 which shows the top and bottom of the chip. Notice the screw holes in the corner, which allow it to easily be disassembled for cleaning.

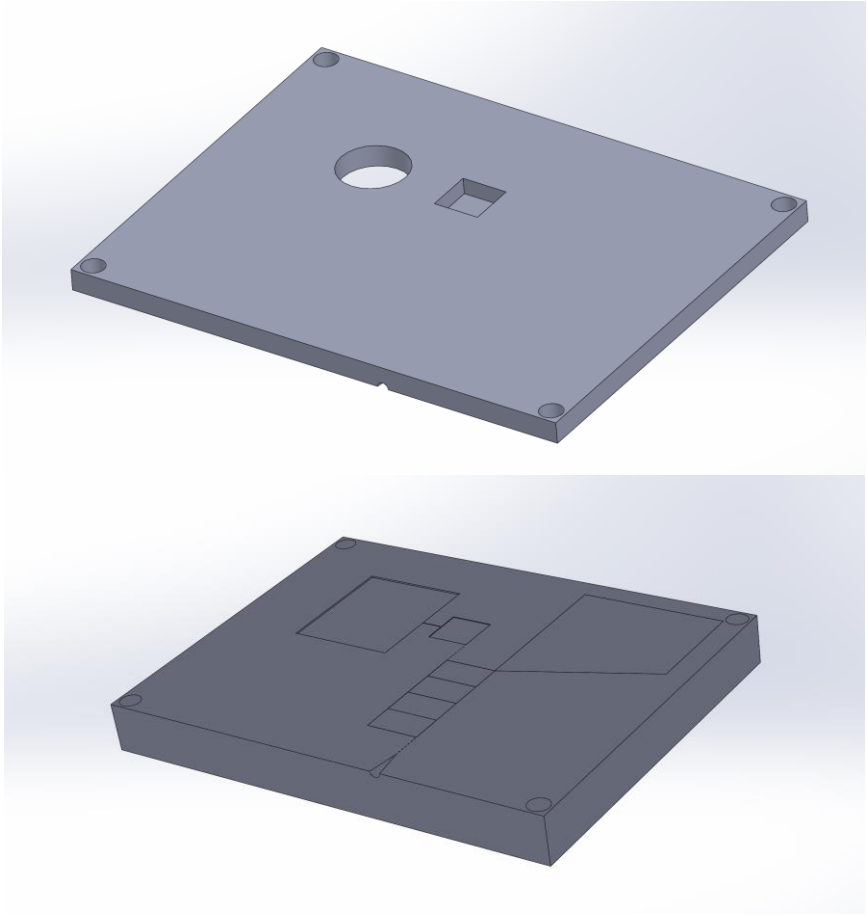

Figure 2: Chip Top and Bottom

RESEARCH DESIGN AND METHODS

FLUID INLET AND SEPARATION

The first component of the lab-on-a-chip is the plasma separation chamber. This chamber consists of the fluid inlet and five bifurcation channels that will filter the plasma out of the blood while the red blood cells gather in a separate tank. This chamber will receive the fluid from the inlet, and fluid will move through the chamber via the usage of a valve actuated peristaltic micropump. This choice of pump was made as these peristaltic micropumps have great control over flow rate, are low in cost, and can be externally attached to the chip. This final point was by far the most important, as it will be much cheaper to produce the chip if only this external pump is needed. The five bifurcation channels will have a slower flow rate than the main channel that the blood enters through. According to the bifurcation law, the erythrocytes, or red blood cells, will travel into the vessel with a higher flow rate, leaving very few cells flowing into the vessel with a lower flow rate. This means that the red blood cells will stay in the main channel and travel to the tank created specifically for storing red blood cells until the chip is cleaned. The plasma, however, will enter these slower flow rate side channels where they are taken to the detection chamber. This can be shown below in Figure 3. This method was chosen as it does not require any mechanical components which would complicate the design of the chip. It was also chosen as filtering the blood into plasma is a necessary step that will make it much easier to detect potential color changes, as if the blood were not filtered it would be impossible to detect the red shift caused by a lack of p24 protein and difficult to perceive the blue shift caused by the presence of p24 protein. Although still not clear, plasma makes it much easier to detect these color changes, and tests will be performed with HIV positive and negative samples to determine exactly what color the solution will become upon detection.

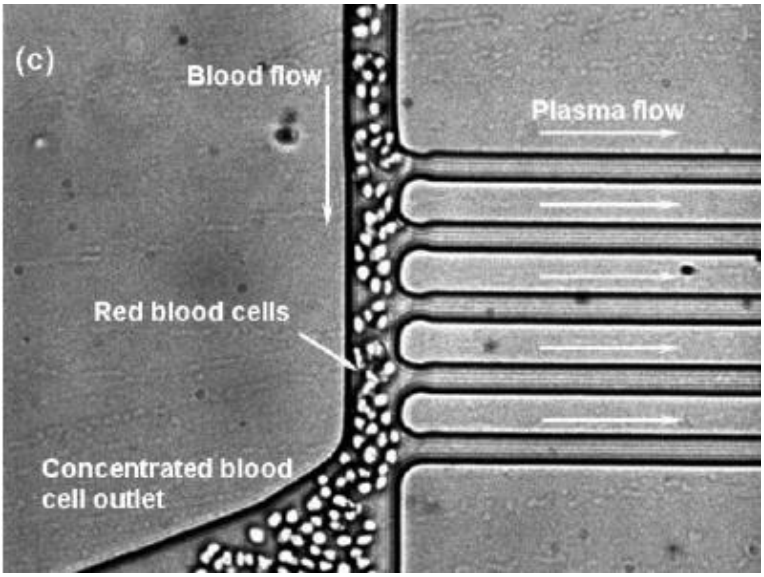

Figure 3: Separation of Blood into Plasma (Yang)

DETECTION CHAMBER

The detection itself works due to the redox reaction between hydrogen peroxide and gold. When this reaction occurs uninhibited, the solution becomes red due to a lack of aggregation of the gold nanoparticles. This will be the case when there is no p24 protein present. However, when the p24 protein is present, it binds with the monoclonal and polyclonal anti-p24 present in the solution. These bindings along with the binding to the streptavidin catalase enzyme result in the decomposition of hydrogen peroxide, lowering the concentration of the hydrogen peroxide. This causes less of the gold to be reduced, leading the gold nanoparticles to aggregate more. This aggregation is what creates the blue color of the solution. This entire process can be seen above in Figure 4.

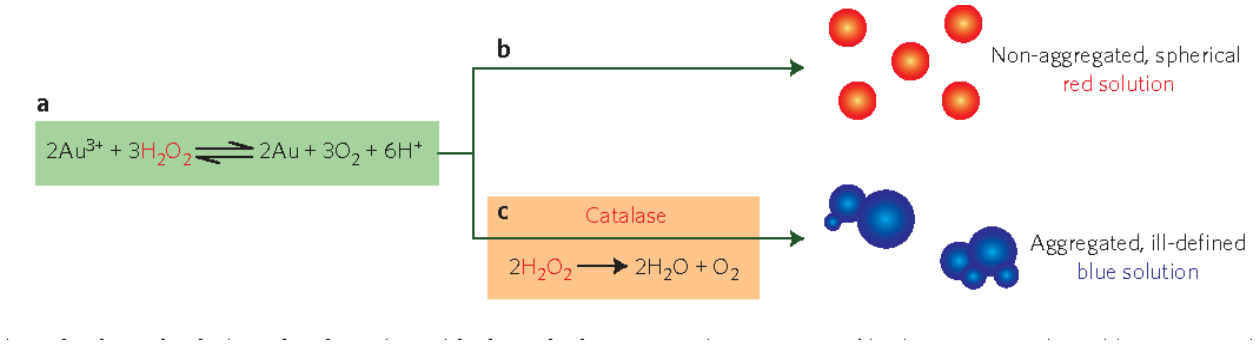

Figure 4: Illustration of Redox Reaction (de la Rica)

The detection chamber consists of a tank where the plasma is gathered, and a tank that will be loaded with the solution of gold nanoparticles, anti-p24, streptavidin catalase conjugate, and hydrogen peroxide before the blood is ever loaded into the chip. There will be hole in the top of this tank to allow for easy insertion of the reagents, which can be done simply using a syringe, pipette, or similar instrument. The reagents themselves will be previously prepared and delivered to the place of use to ensure it is correctly prepared.

A finger pump will be placed in the top of the chip, which will allow for the plasma to manually be pumped into the tank containing the previously mentioned reagents. A finger pump was chosen as it again contains no mechanical components, making washing much easier, and allows for easy movement of the plasma. In order to assure the turbulent flow required, the bottom of the detection chamber is textured. This texturing will lead to turbulent flow as the plasma is introduced to the chamber, which is required in order to ensure proper mixing of the sample with the detection reagents. This method of mixing was chosen as it does not require any mechanical components that would complicate the chip and does not require any action from the user to successfully mix, which will increase the consistency of the chip.

In order to determine the outcome of the test, sheet containing what color changes represent a positive test and what solutions represent a negative test will come with the chip, and the resulting color of the solution will be compared to this sheet to determine whether p24 is present or not. The color change caused by this reaction is significant enough that there will not be any risk of subjective error. Since plasma is not completely clear, testing will need to be done in order to determine the exact colors the p24 positive and negative solutions will appear as. Figure 5 below shows the resulting colors of p24 positive and negative solutions, which are close to the colors that we expect. This method of detection was chosen as it does not require any expensive lab equipment, does not add any mechanical components to the chip that would increase price, and can be detected with the naked eye, making it very easy to use.

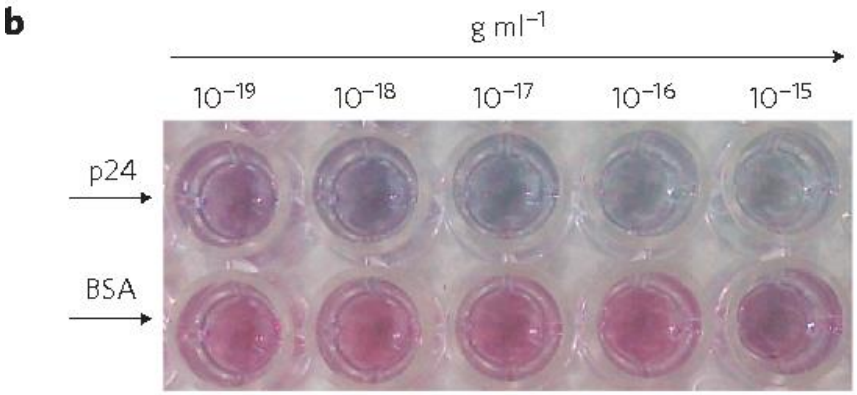

Figure 5: Examples of p24 Positive and Negative Samples (de la Rica)

CHIP FABRICATION

The chip itself will be made of PMMA, which is a clear, hard plastic often used in place of glass. The reasoning for this choice is twofold, as plastic is inexpensive and easy to work with, making it a clear choice, while it is also necessary for the chip to be clear in order to ensure optimal ability to view the color change in the detection chamber. The channels and tanks will be constructed using UV laser microchanneling. This method involves using high energy UV lasers to ablate the surface. This was chosen as it can be used on hard surfaces, which was necessary for this chip, as well as the fact that it is fit for mass production, which is a very important capability. It also has a very low production time meaning the chips would be ready for use quickly. Although this method is expensive, most methods of fabricating microchannels are, and this high cost could not be avoided. Before the channels are constructed however, the base shape of the chip must be constructed, which will be done using traditional machining methods, as it requires nowhere near as much precision. This is the most effective, cheapest method for the basic shape of the chip to be created, which made it an easy choice. The chip will have two parts, a top and bottom. The bottom will contain the microchannels and tanks, while the top will simply cover the channels, and will also contain the hole for reagent to be added as well as the finger pump that pumps the plasma into the reagents. The finger pump itself will have to be constructed within the top of the chip. This will require the use of PDMA instead of PMMA. This is because

the finger pump needs to be able to be pressed by the user, which PDMA permits as it is a softer, more malleable plastic. The finger pump itself will be fabricated using lithography, which allows for the most precise and efficient construction. After the top chip is also completed, the top and bottom will be screwed together. This design was chosen as it allows for the chip to be taken apart for easy cleaning while also making it much easier for the channels to be fabricated.

CHIP TESTING

Finally, the chip will have to be tested before mass production and implementation. First, the concentration of reagents most optimal for detection will be determined. This will be done by observing and recording the color change in various concentrations of the required reagents at various concentrations of p24 presence. Currently, the color change occurs above 1×10^{-18} g/mL of p24. The goal of this stage of testing is to at least maintain, if not improve, this number while also trying to lower the cost of the reagent mixture. The reagent concentrations deemed to be the most effective will then be used in the next stage of testing. This stage of testing will involve obtaining a large number of samples from both HIV positive and negative patients. These blood samples will be put through a prototype chip, and the results of the chip will be recorded. These results will then be compared to results of traditional HIV testing from the same patients, which will reveal any false positive or negative readings given by our chip, and will reveal the overall accuracy of the chip.

FINAL DESIGN

The final design of the chip incorporates a blood inlet, then a main channel that filters the blood and redirects the blood and plasma to their own respective chambers, and then a detection chamber where detection takes place. A model of the bottom half of the chip can below in Figure 6. The top of the chip simply covers the channels, as well as having the finger pump to move fluid from the plasma to the detection chamber and a small hole for easy reloading of the detection chamber.

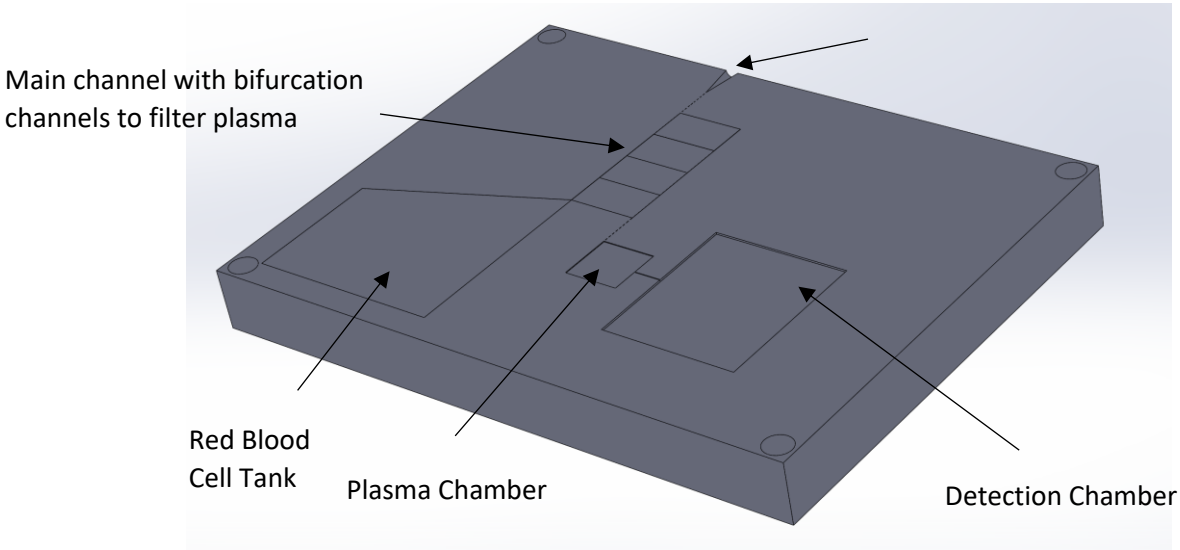

Figure 6: Model of the Bottom of the Lab on a Chip

SUMMARY AND CONCLUSION HIV is an incurable virus that weakens the immune system, leading to eventual death without treatment. With advancements in treatment, early detection leads to a drastically improved outlook for patients. This importance in detection led us to develop a lab on a chip capable of detecting HIV for use in less wealthy regions with less access to testing. Giving these areas access to testing would have a huge effect on the communities in these areas as it could greatly reduce the spread of HIV as individuals have more access to better testing, as well as giving HIV positive individuals earlier access to treatment.

The chip itself will be made of PMMA, and the chip will first be cut using traditional machining methods, then UV laser micromachining will be used to cut the microchannels and all different chambers. The chip will test for the presence of the p24 protein specifically, which is unique to the HIV virion. The design consists of a chamber that filters the blood sample to make plasma via the use of bifurcation channels. This blood will then flow into a chamber, where it will be pumped into the detection chamber using a finger pump. This detection chamber will consist of a solution of gold nanoparticles, monoclonal and polyclonal anti p24, hydrogen peroxide, and streptavidin catalase. When there is no p24 protein present, the solution will turn red, while when the p24 protein is present the solution will turn blue, which will allow for detection.

This chip offers many benefits over other testing methods. The main advantage is that it is a reusable rapid test, so despite having a slightly higher initial investment than some rapid testing methods, will be much cheaper in the long run for communities. Another huge benefit to this chip is its ease of use and accessibility, as it requires very little extra equipment, and the results can be read by almost anybody. Finally, the manufacturing process lends itself to easy mass producing which allows for implementation on a large scale.

With recent advancements in HIV treatments, patients that have been diagnosed early can still have a great outlook. This makes this lab on a chip design even more important, as it allows whole communities to have access to easy, inexpensive, rapid HIV testing. Especially in less wealthy communities, this lab on a chip can give patients a much better outlook and the chance to seek treatment much earlier.

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