APP N15-2

Engineering 1282H

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Team Y4

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SPECIFIC AIMS

Human immunodeficiency virus 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, however these advancements are not as ubiquitous in countries with less access to medical resources. Along with this, early detection of HIV is critical in reducing the damage caused by this virus. Because of this, 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 will be deployed across impoverished areas. This will allow for the sought-after early diagnosis of HIV that will allow individuals currently with little access to testing to be able to seek treatment earlier.

Our lab-on-a-chip design relies on multiple previous discoveries which we have combined. Firstly, the separation of blood into plasma using multiple bifurcation channels is be utilized to separate blood. This is necessary to detect the color change in the next discovery, which is that a solution consisting of gold nanoparticles, monoclonal and polyclonal anti-p24, hydrogen peroxide, and streptavidin-catalase conjugate will change color in the presence of the p24 protein. This is due to the reduction of the gold ions with the hydrogen peroxide occurring at different rates based on the presence of p24 solution. This change in reduction rates leads to the gold nanoparticles aggregating differently, which subsequently leads to the observed color change. We propose the development of our proposed chip that will provide higher ease of use at a lower cost in comparison to traditional testing for use in low income areas. The following aims will lead to the best possible product that follows 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 then attempt to seek health care if required.

Aim 2. To optimize the design of the chip, including materials, pumps, and design features used as well as reagent amounts used in detection in order to minimize cost. As the goal is for this chip to be employed in low-income regions worldwide, it is important to minimize the cost of both the chip and its required reagents. Therefore, the quantity of expensive reagents must be minimized while still providing an accurate test.

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 ways to pre-mix large amounts of the testing reagent required. Methods of distribution, target areas, and possible distribution partners will also need to be determined.

BACKGROUND

Intro disease analyte – p24 protein on the HIV virion surface

Past studies - Western Blot Tests

Serological Testing

Enzyme Immunoassay

Key anatomy - protein is located on the virion and is the key biomarker that interacts with the gold nanoparticles

Establish built in technology - Valve actuated peristaltic micropump

Bifurcation channels

Finger pump

Gold Nanoparticles

Hypothesis- Color change for easy of detection of presence of disease

Overview – Significance and Application

Innovation

Research

Methodology of LOC

Conclusion

SIGNIFICANCE

Why this disease – Ease of diagnosis in areas not easily accessible to labs

Current detection methods – HIV screening: Antibody and antigen screening that can detect the disease 18-45 days after exposure

Require a laboratory setting to run the antibody cell count which is not readily available to rural areas

LOC – portable

Cost efficient

Rapid response time

Call to action/relevance/benefits - Early detection allows for treatment to start immediately and maintain the remaining immune system

Prevent the spread of HIV to others – unborn babies

HIV destroys the CD4+ cells that defend the body from minor illnesses and diseases and the body is left without an immune system

If left undetected, can result in AIDS

INNOVATION

How is it new technology - Reordering of old methods and concepts

New technique or old methods in different order - Different order

How is it different from other detection methods - Allows the user to view the test result

No need for samples to be sent to a lab for testing

Immediate test result due to change of color

RESEARCH DESIGN AND METHODS (Will have to add information/depth for final version)

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 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.

DETECTION CHAMBER

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. The person conducting the test will then gently shake the chip in order to ensure mixing of the sample and reagents and will then observe the solution and look for the color change. A 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. 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.

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 be have two parts, a top and bottom. The bottom will contain the microchannels and tanks, while the top will simply close 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 top and bottom will then 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. This will be done by 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. Testing should also be done to optimize the test itself in order to limit the amount of reagent required, specifically the more expensive reagents. This will be done similarly by comparing the results of a prototype chip to traditional testing using decreasing amounts of reagent. The test that is most accurate while using the least expensive combination of reagent amounts should be used, as it will provide the most accurate test at the cheapest cost, which is a very important aspect of the chip.

CONCLUSION

Summary – Purpose of research and LOC

Effect of LOC on community

Construct LOC

Optimize design of LOC

Method of production

Key design features – Bifurcation channels

Gold nanoparticles

Benefits - Reusable

Cheap

Mass produced

Accessible

Call to action - disease that has no cure so the next best thing is to produce the ability to easily and simply diagnose a patient with HIV

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