Optimization and Adaptation of a Magnetic Based Microfluidic Immunoassay

Eliza Cricco-Lizza  
cricco.lizza@gmail.com

Courtney Hollenberg  
courtney.hollenberg@gmail.com

Alicia Lee  
allee94729@gmail.com

Iyore Olaye  
iyoreolaye@gmail.com

Ariana Schanzer  
arianaschanzer@gmail.com

1. Abstract

Microfluidics is a dynamic and constantly developing field involving experiments that concern fluids at the micron level. A three-layer microfluidic immunoassay device was produced and analyzed for optimum efficiency at the Rutgers University Biomedical Department BioMEMS laboratory. Currently, the device has a tendency to clot, and the magnetic substrate drags along the channel walls, causing an inconsistent incubation time. By revising the placement and the design of the magnets in the device, the velocity of the flow was increased, improving the overall process. To eliminate the disadvantages of the magnets, purely hydrodynamic versions of the device were designed for future fabrication.

2. What is Microfluidics?

After the Cold War, the Defense Advanced Research Projects Agency (DARPA) of the United States Department of Defense formed a series of programs to develop a field deployable microfluidic system designed to detect chemical and biological threats [6]. Since the 1990s, this area of research has continued to develop.

Microfluidics comprises the study and experimentation of fluids in channels that are on the micrometric scale. The volume of fluid that passes through devices containing these micro-channels is of a magnitude between $10^{-9}$ and $10^{-9}$ liters [6]. Hence, only a small amount of reagent is used to produce reliable results.

The small scale of these fluids gives them unique physical properties and opens up a whole new field of study with diverse applications and many advantages [6]. For example, microfluidics can be used to screen conditions for protein crystallization, to perform chemical synthesis and to separate cells. The small size of a microfluidic system also allows it to be combined with various other processes to create a miniature “lab-on-a-chip”. Multiple functions can be conducted simultaneously on the same sample in one “lab-on-a-chip”, drastically reducing the time needed to produce clinically relevant data. These miniature labs are portable and easy to use; untrained personnel (such as clinicians, police officers, or public health officials) can use them to quickly and accurately conduct sophisticated analyses. Clearly, there is great potential for use of these “labs-on-a-chip” at home, in the field, at the scene of an accident, or in any situation where rapid detection of biomarkers is necessary [10].

This tiny device can perform a range of functions thus referred to as a “lab on a chip”
3. Physical Properties of Microfluids

Unlike macrofluids, whose turbulent flow contains perpendicular currents and swirls, microfluids move through laminar flow. Therefore, the velocity, pressure and other flow properties of the fluid are constant at each point, and the flow seems to consist of thin layers of fluid all parallel to each other. Laminar flow in a straight pipe may be considered as a set of concentric cylinders of fluid, the outside one fixed at the pipe wall and the others moving at increasing speeds as the center of the pipe is approached [4].

A Reynolds number (see appendix 1) is a dimensionless number that relates inertial forces to viscous forces. If the Reynolds number of a given flow is less than 2000, the fluid is undergoing laminar flow and contains one of the main properties of microfluidics.

The feature described by the Reynolds number, as well as cohesion and adhesion, capillary action, and lack of inertial forces are the properties behind the device’s distinct abilities [4].

4. Enzyme-Linked Immunosorbent Assay (ELISA)

An immunoassay is a biochemical test to measure the presence or concentration of a specific biomarker in the body. A heterogeneous enzyme immunoassay, also known as enzyme-linked immunosorbent assay (ELISA), is a typical technique used in immunology to detect the presence of an antibody or antigen in a sample. ELISA tests can be of the competitive or “sandwich” type. In a competitive assay, the antigen in the unknown sample competes with a labeled antigen to bind with antibodies. In sandwich assays, antigen in the unknown sample is bound to the antibody site, then labeled antibody is bound to the antigen. This study focuses on a sandwich-type assay. In the double antibody sandwich technique, a solid is coated with a specific antibody that is then reacted with the antigen-containing test sample. Then, enzyme-labeled specific antibody is added, followed by an enzyme-substrate that produces a visible signal and allows for measurement of the amount of antigen [5].

Diagram of double antibody sandwich

The indirect method of ELISA is distinctly pertinent in studies of infectious diseases where there is often a need for measurement of antibody, rather than antigen. In this method, the antigen is immobilized onto a solid phase. A serum is added, and any specific antibody attaches to the antigen. After washing to remove unreacted serum, the antigen-enzyme conjugate attaches to any antibody that has already fixed to the antigen. After another wash to remove unreacted material, an enzyme-substrate is added, which commonly results in a color change that allows the amount of fixed conjugate to be measured. This color measurement is proportional to the antibody level in the test sample. Various other modifications of ELISA have been used, but these two are the most common [5].
5. Benefits of using MF for Immunoassays

Microfluidics can continuously measure any protein biomarkers from any biofluid source, thus it is a perfect method to conduct immunoassays in a simpler fashion than the ELISA technique. Superior control and accuracy allow microfluidic systems to process data and execute operations more quickly than conventional macroscopic systems. At the same time, much smaller amounts of samples, chemicals, and solvents are consumed, thereby minimizing costs [3]. This reduced sample size allows for a continuously flowing immunoassay monitoring system that can produce data at multiple time points throughout various processes [10]. Thus, concerns about the quantity of sample needed to monitor subjects and its effects upon their health are minimized. For example, using conventional immunoassays to analyze children's conditions during pediatric cardiac surgery is very risky. A child undergoing cardiac surgery is in critical danger from massive blood loss and cannot spare the blood needed for conventional immunoassays. However, microfluidic immunoassays require only micro-liters of blood sample and consequently pose no threat to children undergoing life-threatening operations [1,11]. The ability to continuously analyze various processes through microfluidic immunoassays will allow scientists to study different reactions, improve treatment techniques, and develop new medicines.

6. Methods

Initially, microfluidic devices were cast in silicon, glass, or metal, which offer excellent chemical and physical stability, solvent compatibility, and optical and surface properties [10]. However, devices fabricated in glass or silicon are extraordinarily expensive and are therefore impractical for exploratory research in
microfluidic systems. Silicon, metal, and glass devices are usually unnecessary or inappropriate for analyses of biological samples in water; none of these materials possess the properties (especially permeability to gases) necessary for work with living mammalian cells. Additionally, silicon is opaque to visible and ultraviolet light and consequently cannot be used with conventional optical methods of detection. It is also difficult to fabricate the components required for microanalytical systems in rigid materials. The high price and inflexibility of silicon, glass, and metal have driven a movement towards alternative materials, most notably the polymer polydimethylsiloxane (PDMS). PDMS is an optically transparent, soft elastomer that possesses good gas permeability, allows for easy testing of new concepts, and supports certain very useful components, such as pneumatic valves [6]. Compared to metal, glass, and silicon, PDMS is cheap and easy to manipulate; consequently, many labs, use PDMS to make microfluidic devices.

The three layers of the device are each made independently using a distinct process. The PDMS is a two-part heat-curable system that is mixed in a 10:1 ratio with the included cross linker. Enough PDMS was poured to fill the master template to the desired level (1 gram of PDMS for every 1 mm of thickness). The mold was then placed in a vacuum chamber for 10 minutes to remove the gas bubbles in the PDMS, which could interfere with the device's performance. Once the bubbles were removed, the PDMS-filled master was cooked in an oven at 100°C for an hour in the completely sterile clean room.

After the PDMS was cured, a sharp razor blade was used to cut and peel out the PDMS, which was solid, out of the master. Hollow needles of various diameters were used to punctured input and output holes straight down through the PDMS for insertion of tubing. Smaller punctures were made at distinct locations to allow the movement of fluid between layers of the device. The solidified PDMS was cleaned with acetone and distilled water and dried with pressurized air, in order to remove oils, dust, and various debris. Once the PDMS was as clean as possible, it was ready to be bonded with either another piece of PDMS or a glass slide. The cleaned surfaces were exposed to a corona discharge, an ionization gun that activated the surfaces and allowed them to bond. The bonded device was placed in a second oven at 65°C and after 45 minutes, the PDMS was cured [2]. Once each of the three layers was separately made, they were bonded in order to create the complete device. The first layer and second layer were exposed to the corona discharge and bonded together. The newly bonded first and second layers were put into the 100°C oven to cure. Once cured, the process was repeated and the second layer was bonded with the third layer, which was already bonded to the glass, to complete the microfluidic immunoassay device, and it make it ready for use.

For use, the device was flushed with a phosphate-buffered solution (PBS) or de-ionized water in order to clear out debris as well as check for leaks that may have formed between the PDMS and glass. Then, the fluids and beads that were used for analysis by the device were prepared for processing. A twenty micro liter portion of beads was first diluted with five hundred microliters of a PBS. The fluid was then placed in a vortex in order to suspend the beads in the solution. This solution and sample fluid to be tested were placed in syringes and attached to tubing.

The fluid was pumped to introduce the magnetic beads to the device. A banner then formed in order to ensure that equilibrium was reached. In order for the device to perform its function, however, a magnet needed to be placed on the top of the device to allow the transfer of beads from one fluid to another.
7. Focus/Purpose

After spending ample amounts of time studying the microfluidic immunoassay device, there were many identifiable changes that could be made in order to accelerate the speed at which magnetic beads moved through the channels. The previous device template consisted of one magnet that sat in the center at top of the device. The purpose of the magnet as discussed in the introduction, is to attract the centroid of the magnetic bead. The location of the centroid of the magnetic bead determines how a bead moves done the channel referring to its position. Whether the bead moves along the top of the channel, the middle, or the bottom of the channel is determined by the magnet and its placement. The location and position at which the bead moves through the channel alters the beads speed.

The immunoassay device favors rapid movement of beads. A rapid bead speed is crucial in the prevention of bead clots in the channel of the device. Clots are detrimental to the immunoassay and the substance that is being tested. Flaws in the device and foreign particles that occasionally get trapped in the channel cause the clots. These irregularities are often cracks in the PDMS, the substance that the device is made of. Usually, the particles that cause clots are hair particles.

The faster the bead moves, the more likely it is to bypass the foreign particle or crack and avoid the clot. When moving at a slow pace the beads tend to entertain the foreign particles or crack. Consequently, they get stuck around it, and are unable to pass the channel interruption. As one beads becomes stumped and paused around the clot agitator, the beads after it follow suit. This action produces a clot. The beads move at an increased speed when the centroids of the beads flow down the center of the channel.

The original orientation of the magnet allows the bead to travel along the top of the channel avoiding the center. This orientation allows the bead to move through the channel, but prevents it from moving at the pace needed in order to avoid clotting. It was hypothesized that by increasing the number and location of magnets on the device, the position at which the beads moved through the channel could be altered. As a result the bead velocity might also be increased. The centroid of the bead is contained at the bottom of the liquid. The centroid holds the bead down and permits it to move through the channels with the fluid. In theory the magnetic forces from the magnets will attract the beads. Having magnets on the top of the channel will pull the bead upward defeating the downward force of the fluid. In essence, having magnets at the bottom of the channel will balance out all of the forces.

8. Results

The magnets were placed in various positions in order to evaluate its affect on the bead flow.
The standard orientation was a magnitude five magnet placed at the top center of the device.

In trial one, two size three magnets were placed at the bottom of the device. One size five magnet was placed at the top of the device. There was an insufficient amount of force to remove the bead from the adhesion to the wall. Thus the beads continued to move at the top of the channel at a continuously slower pace.

Trial three involved orienting the magnets in an inverted triangular shape. Two size four magnets were placed on the top and one size four magnet at the bottom. The two size four magnets at the top were unable to pull the beads upward, resulting in a loss of beads down the waste channel of more than ninety percent. When larger magnets were used the magnetic force was too great causing the beads to slide along the top walls of the channel, thus slowing their velocity.

In trial two, two size four magnets were used at the bottom of the device instead of size three and one size four magnet was at the top center. This caused over eighty percent of the beads to travel down the waste channel. The orientation hurt the functionality of the device. A smaller magnet at the top of the device was unable to pull the beads out of the lower flow from the bottom of the channel.

Trial four involved 5 magnets, four size four magnets at each of the corners and a size five magnet at the top center. This setup failed because there was not a balance of forces. The downward pull was significantly greater than the upward pull causing an ample amount of beads to go down the waste channel. This
9. Discussion

Increasing the flow rate of the microfluidic immunoassay helps troubleshoot clots and provides quicker results. This is one step towards improving the overall device.

Though separating the beads through use of inserted magnets has proven sufficiently effective, another idea has been proposed known as “pinch flow.” If successful, this method would both reduce cost and improve the stability of the device. In this process, a single channel contains three liquids in one layer of the PDMS. Instead of magnets attracting the beads upward, the buffer solution containing the beads flows into a channel with two other liquids: the sample and distilled water. The distilled water flows fastest while the buffer solution moves with the least speed. The distilled water is made to flow fastest so that it monopolizes space in the channel and pinches the other two liquids closer together. As the height of the buffer solution decreases past the length of the bead’s radius, the centroid of the bead (or its center of mass) shifts from the buffer solution to the sample solution. Because the bead will travel only in the solution where its centroid is contained, the bead has effectively been transferred from the buffer to the sample and will flow out of the joint channel into one containing only the sample solution. [8].

Using this method over the magnet process is much preferred because it significantly

Trial 5

Therefore, in trial five instead of having two medium magnets at the bottom one magnet was placed at the bottom center. Three size four magnets were placed along the top of the device, one in the center and two in the corners. The forces proved to be balanced. However, the pull, from the magnet on upper left side of the device, caused the beads to slow down when moving through the incubation spiral. The magnet was placed over the spiral, making this setup faulty.

Trial 6

Trial six proved to be optimal. It consisted of a three size four magnets, placed in the upper right corner, top center, and bottom center. The bead flow through the device was at a very high velocity in both the channel and incubation spiral. The beads moved down the center of the channels avoiding clots, and very few beads moved down the waste channel.
reduces the cost and increases the effectiveness of the device. Not only would the device become a single layer of PDMS instead of three, but also the cost of the magnets of the original would be completely eliminated. The device’s robustness, or stability and strength of function, would also be improved because the process occurs over a more controlled and limited area. The beads are no longer being directed based on the distant pull of a magnet, but on the more reliable properties of liquids. Clots are much less likely to form in the device and, in general, the process will perform with more stability and consistency.

9.1 Designs of Devices with Pinch Flow

There are many different ways to design a device that employs pinch flow. One method would be to form one channel that snakes back and forth across the length of the device. Here the beads would have a significant distance to travel and as such enough time to separate and incubate. The more winding turns present in the device, the lower the chances that a clot will form.

Another way to implement this idea is through horizontal rows of vertically snaked channels. The vertically snaked channels would allow for more space on the slide, and compliments the idea that all three layers of the current device could be condensed onto one.

The channel can also be made to constantly alternate in width in order to keep the beads moving. In the wider portions of the channel, the liquids will travel more slowly, but once they reach the thinner areas, they will speed up. This follows the equation of continuity which states $a_1v_1 = a_2v_2$, $a$ representing the area and $v$ the velocity. In this way, the beads speed up and slow down, kept in constant fluctuation so they can continue moving throughout the device.

Another proposed design would involve altering the current shape of the incubation spiral. The original microfluidic immunosay device contained circular incubation spirals; the new design includes triangular incubation spirals that are rounded at the corners. The triangular spirals would actively decrease the chance of clotting of the beads in the device. Normally when a clot occurs it is very difficult for traveling beads to avoid it. Because the beads must travel through the clot without getting caught, it is very difficult and majority of the beads end up getting caught and contribute to the growing of the clot. With a triangular spiral, if a clot occurs at the point or outer edge of the spiral, the bead can go around the clot instead of going through the clot. Thus more beads can avoid the clot instead of being trapped.

These designs are ready for fabrication and testing and can lead to an even greater improved device.

10. Conclusion

The microfluidic devices discussed in this paper are currently being explored with a focus on cardiovascular application. In the future, the conclusions found from this research may be applied to diseases involving all areas of the body because the device is capable of detecting any biomarker quickly and efficiently.

Eventually, this model will be converted into a glass device that can hopefully enhance current medical procedure significantly. Each improvement in the structure of the microfluidic device contributes not only better understanding of the process, but also a more efficient method of performing it.
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12. References


13. Appendix

1. Reynold’s Number Equation:

\[ Re = \frac{LV_{avg}}{\mu} \]

where \( L \) is the most relevant length scale, \( \mu \) is the viscosity, \( r \) is the fluid density, and \( V_{avg} \) is the average velocity of the flow.