Combining Electrowetting and Magnetic Bead Manipulations for Use in Microfluidic Immunoassays

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Abstract
This experiment investigated reducing the cost of microfluidic immunoassays. Magnetic microbeads are used in these assays to increase the efficiency of immunoassays, but they also raise the cost. This experiment aims to reduce the number of magnetic beads necessary by using electrowetting in conjunction with the beads. Single and parallel plate electrowetting devices were tested individually and the magnetic microbeads. The devices did not succeed in separating the beads from the droplets, but the results show many promising options for further investigation.

1. Introduction
With the increasing pace of today’s society, current medicine requires fast and accurate diagnoses for patients. One way of making diagnoses is through immunoassays, which are becoming very prevalent. An immunoassay is a type of test that involves screening biological samples to determine the concentrations of various antigens. These allow doctors to make more informed decisions regarding the patient’s treatment. ELISA (enzyme-linked immunosorbent assay) is a common type of immunoassay. It requires a blood sample size on the scale of mL, which is unsuitable for infants and immunocompromised patients who cannot afford to lose large amounts of blood. It also takes 2 days of incubation time, which places a risk on patients with urgent health concerns. Technologies in microfluidics have created a different type of immunoassay that uses magnetic microbeads. It can be performed within minutes with only 5-10 µL of blood, making it safer for patients who cannot afford to lose blood, and more efficient for doctors. However, this process is very expensive due to the large number of microbeads needed. During the assay, the microbeads need to be separated from each sample and pulled through microchannels into a new solution. In order to separate, the microbeads need to break the surface tension of the sample, and this requires thousands of microbeads.

This project aims to create a method of performing the immunoassay with fewer microbeads, therefore reducing the price. The microbeads cost $852 for a 10 mL vial, so the current method of testing is economically unfeasible for widespread use. To reduce this cost, it is proposed that magnetic actuation be combined with electrowetting. Electrowetting is a technique that manipulates liquid using electricity to reduce the contact angle between fluid and a solid surface, thus reducing the surface tension. One of the roles of the magnetic beads in the immunoassay is to break the
surface tension of the sample fluid. Reducing the surface tension with electrowetting means that a smaller force is required, and therefore fewer beads are needed to complete the same task. The successful combination of magnetic actuation and electrowetting can lead to a smaller microbead requirement and a more affordable microfluidic immunoassay.

2. Background

2.1 ELISA

ELISA (enzyme-linked immunosorbent assay) is a test used to identify and quantify specific antigens in a sample, and it is often used to monitor antigen concentrations in blood samples. The mechanism for ELISA utilizes antibodies specific to the desired antigen. Primary antibodies in wells are immersed in the sample, and the antigen in the sample binds to this antibody. The wells are then washed, and a solution containing a secondary antibody is added, and the secondary antibody binds to a different epitope of the target antigen. This secondary antibody has a fluorescent or a colorimetric enzyme tag that is used to determine the concentration of antigens in the sample. This tag is easily measurable and correlates to the concentration of the substance of interest. ELISA is traditionally performed in a 96 well plate, which requires 50 µL of blood per well and a minimum of two days in a laboratory to process the test.

2.2 Immunoassays and Microfluidics

Microfluidics is the study, design, and creation of systems dealing with the flow of small volumes of liquid. In recent years, microfluidic techniques have been combined with magnetic beads to create a new method of performing immunoassays. These tests reduce the amount of blood and time required for medical procedures. First, magnetic microbeads are conjugated with an antigen-specific monoclonal primary antibody and placed in a well containing a blood sample so the primary antibodies bind with the target antigen. Then, the beads are pulled with a magnet through a microchannel containing oil into a different well. The new well contains a secondary antibody that binds to a different epitope of the target antigen. The secondary antibody is then bound to a fluorescent or colorimetric tag that is used to detect the concentration of antigens found in the blood sample.

![Fig. 1. Diagram of microbead in microfluidic immunoassay](image)

This type of antigen capture and detection is very similar to the process used in a traditional ELISA, but the microfluidic method uses only 5-10 µL of blood, which is ideal for infants and immunocompromised patients that cannot afford to lose large quantities of blood. The microbeads also create a large surface area-to-volume ratio to ensure maximum antigen capture. In addition, their magnetic properties allow a separation system in which magnets can pull the microbeads from one sample to another through microchannels. This process creates a series of incubations within the device and therefore it increases the speed of the wash steps necessary in an ELISA. This means that the immunoassay can be performed at much faster speeds, which is more practical for use in surgery or other urgent situations. In addition, there is a possibility of developing a continuous flow test. It will produce results at even faster speeds, and it will require very little blood from the
patient. One application of this type of immunoassay is surgery. A continuous flow test will allow surgeons to monitor biomarkers continuously, which is important for procedures such as cardiopulmonary bypasses (CPB). The ability to detect inflammatory biomarkers in the blood will alert surgeons to problems earlier, which will reduce complications and increase recovery speed.⁶

![Diagram of slide used to conduct the microfluidic immunoassays. First, the beads are ejected from the reservoir with a magnet, then they are transported through the channels, and then deposited in the adjacent well.](image)

**Fig. 2.** Diagram of slide used to conduct the microfluidic immunoassays. First, the beads are ejected from the reservoir with a magnet, then they are transported through the channels, and then deposited in the adjacent well.⁵

![Magnetic beads being pulled through a channel in a microfluidic immunoassay](image)

**Fig. 3.** Magnetic beads being pulled through a channel in a microfluidic immunoassay⁶

### 2.3 Magnetic Beads

The magnetic microbeads used in these microfluidic devices are superparamagnetic. Superparamagnetic substances have magnetization that randomly flips as temperature varies, leading to a higher magnetic sensitivity than typical paramagnetic substances. This means that the superparamagnetic beads react quickly when a magnetic field is applied, and in addition, they do not agglomerate after the magnetic field is removed.⁹ In addition, the addition of microspheres to a solution reduces the viscosity of the overall solution, since the fluid molecules interact more with the beads than with the walls of the microchannels. A low viscosity fluid requires less energy to flow, which is ideal for small systems. The magnetic beads in this experiment are composed of a magnetite (Fe₃O₄) core, and a streptavidin coating.⁹ In microfluidic immunoassays, thousands of beads are required to break the surface tension of the fluid and eject a bead droplet from the sample, as seen in Fig. 2. Since the microbeads are too costly, this number needs to be reduced to make the test economical.⁴

### 2.4 Electrowetting

Electrowetting-on-dielectric is the process of changing the wetting properties of a surface by applying an external electric field.¹¹ If a droplet of conductive liquid is exposed to an external electric field, the distribution of ions within the droplet changes, and as a result the shape of the droplet adjusts to maintain equilibrium. As a higher charge is applied, the droplet becomes more wettable, and therefore the contact angle of the droplet decreases. The contact angle of a droplet is described by Young’s equation.

\[
\cos \theta_Y = \frac{\sigma_{sv} - \sigma_{sl}}{\sigma_{lv}}
\]

In this equation, \( \theta_Y \) is the contact angle between the droplet and the solid surface, \( \sigma_{sv} \) is the surface tension between the solid and the surrounding vapor, \( \sigma_{sl} \) is the surface tension between the solid and the liquid, and \( \sigma_{lv} \) is the surface tension between the liquid and the vapor. When a voltage is applied, the change in contact angle due to electrowetting is described by the Young-
Lippmann equation.

\[ \cos \theta = \cos \theta_Y + \frac{\varepsilon_0 \varepsilon_d}{2 d \sigma_w} U^2 \]

In this equation, \( \theta \) is the new contact angle, \( \varepsilon_d \) is the dielectric constant of the insulator, \( \varepsilon_0 \) is the dielectric constant of the liquid, \( d \) is the distance between the electrodes and the water droplet, and \( U \) is the voltage applied. As the voltage is increased, \( \theta \) decreases, which decreases the surface tension between the liquid and the solid, making it easier to actuate the droplet.\(^{12}\)

This is beneficial, because since PBS is isotonic with blood, converting from water to a biological fluid for use in an immunoassay will not be difficult.

In order to actuate a droplet, it has to bridge two or more electrodes. For a parallel-plate configuration, a second plate is placed on top of the first one and either air or an immiscible liquid surrounds the droplet. This configuration makes the droplet thinner, further increasing the control over the droplet’s surface tension\(^{14}\) and consequently the liquid’s ability to move, split, and merge.\(^{15}\)

**3. Procedure**

**3.1 Device Fabrication**

The template for the electrode pattern necessary for the microfluidic device was created using AutoCAD, a computer aided design program, and patterned onto a glass microscope slide through photolithography, a process that transfers a design onto a substrate. The design created in CAD was printed onto a film mask, which would later be used in the exposure phase of photolithography. The resulting plate formed the basis of the microfluidic device.

The process of photolithography required 4 steps.\(^{16}\) First, a glass microscope slide was soaked in acetone, isopropanol, and deionized water for ten minutes each to remove contaminants. The slide was then baked to assure that the slide was completely dry. In the next step, a positive photoresist, which degrades when exposed to UV light, was spin coated onto the slide. The third step consisted of the softbake, in which the plate was heated to cure the photoresist and prevent bubbling. In the next step, a photolithography system was used to expose the photoresist to UV light in the pattern defined by the AutoCAD mask. Then, the slide was placed in a developing solution, causing the areas that were exposed to the UV light to degrade.
Fig. 6. Process of fabricating the electrode plates

After the photolithography process, the photoresist was hardened with the postbake. Next, hydrofluoric acid (HF) was used to etch the exposed glass so that the platinum used to make the electrodes can be effectively deposited and bonded to the glass. The HF does not affect the photoresist, so it only etches onto the exposed glass in the shape of the electrodes. Then, platinum was sputtered onto the slides using a physical vapor deposition system. The platinum only binds permanently to the places where the glass was etched with HF. Finally, the slide was rinsed with acetone to remove the photoresist and the excess platinum, so that only the platinum electrodes remained in the glass slide.

Fig. 5. Top: Photolithography mask created in CAD
Bottom: Resulting slide after the photoresist developed

In order to perform electrowetting, the slides were coated with Cytop, an amorphous fluoropolymer that forms a hydrophobic barrier between the electrodes and the water droplet. First, the slides were placed in the plasma generator at 100 W for 60 seconds in order to activate the surface to improve bonding. Next, 250 µL of Cytop was pipetted onto the slide and spun using the spin coater for 30 seconds at 3000 rpm. In order to cure the Cytop, the slides were then placed on a hotplate at 100°C for 90 seconds and placed in a 150°C oven for 2 hours.

Single plate electrowetting only requires the electrode slide, but in order to perform parallel plate electrowetting, a separate device must be built. The electrodes that were used in the single-plate electrowetting formed the base of the device. Then, a gasket was created out of PDMS (polydimethylsiloxane). The PDMS and a hardener were mixed in a ratio of 10:1, and then 1.5 ml was poured onto a clean petri dish. Then, the dish was placed in a desiccator for two hours to remove the air bubbles from the solution.
After it hardened, the PDMS layer was cut so that when it was placed on the electrode slide, the electrodes were still exposed. Finally, an ITO (Indium tin oxide) slide was coated with Cytop using the same procedure as before. ITO is a conductive material that is spread onto a glass slide, and it is used to apply ground to the top of the water droplet. As with the platinum electrodes, the ITO also needs a layer of Cytop to insulate the water droplet from the conductive material. After the ITO slide is coated, it is placed on top of the PDMS gasket, leaving the wire-connected electrodes uncovered. An edge of the ITO slide was also left uncovered, and then the layers were secured with tape.

3.2 Single Plate Electrowetting

In order to test the effectiveness of the electrodes, single-plate electrowetting was used. In this procedure, 2 µL, 4 µL, and 6 µL droplets of deionized water were tested. In addition, phosphate-buffered saline (PBS) was tested in the same volumes. The droplet being tested was placed so that it bridged two electrodes, and wires connected to a DC power supply were touched to the corresponding electrodes in order to make the droplet move from one electrode plate to another. Applying a positive charge to one electrode and grounding an adjacent one made the water droplet move towards the positive voltage. The DC voltages were tested at 40 V, 60 V, and 80 V.

Single plate electrowetting was also tested using a non-conductive top plate to reduce the contact angle of the droplet. A PDMS gasket was placed on top of the original electrode plate so that it did not cover the electrodes, but so it created an elevated platform for the top slide. The water droplet was placed between the two slides, which flattened the droplet and reduced the contact angle. The electrowetting was then conducted using the same procedure as the single-plate electrowetting without the top slide.

3.3 Parallel Plate Electrowetting

Parallel plate electrowetting is a process used to split droplets. This is useful for microfluidic immunoassays because the
beads have to separate from the sample droplets in order to go through the channel to the next well. The parallel plate device consists of an electrode slide, a PDMS gasket to support the top slide, and an ITO slide that covers a water droplet. In order to test this technique, 3 wire leads were needed. The ground lead was attached with an alligator clip to the ITO top slide. The water droplet was placed in the center of the electrode plate, and the two voltage leads were touched to each adjacent electrode. The droplet will be pulled towards each positively charged electrode, and with sufficient elongation, the surface tension will break and the droplet will split in half.

3.4 Electrowetting and Magnetic Beads

Testing Magnetic Bead Manipulations

A solution for testing the effectiveness of the microbeads was made with 4 parts water and 1 part bead solution. The small vial containing the solution was first placed in a vortex mixer to combine the beads with the water solution, and to excite the microbeads. A 4 µL droplet of the solution was tested on a single plate that was elevated, and a magnet was placed under the plate so that it could move and affect the magnetic beads. A 10 lb. pull strength magnet and a 5 lb. pull strength magnet were both used. Next, the magnets were tested in conjunction with single-plate electrowetting. A 4 µL droplet of the bead solution was placed on an elevated electrode slide, and a magnet was used to aggregate the beads on one side of the droplet. At the same time, the procedure for single-plate electrowetting was used to pull the droplet in the opposite direction, with the goal of separating the magnetic beads and the water droplet.

4. Results

4.1 Single Plate Electrowetting

The 2 µL droplet was too small to bridge the electrodes used, so it could not move. The 4 µL droplet was the best volume for this experiment. It was big enough to bridge the electrode plates and small enough to move quickly with the applied voltage. The 6 µL droplet was also big enough to bridge the plates, but since it had a larger volume, it took more energy to move it easily. The deionized water and the PBS worked equally well. For the voltages tested, the higher voltages yielded more movement from the droplets, but they also raised the risk of inducing hydrolysis as the Cytop coating failed and the water droplets came into direct contact with the electrodes. A voltage of 40 V was too small to induce any movement from the droplets because it did not reduce the contact angle enough. A voltage of 100 V was too large, as it created a lot of movement, but hydrolysis occurred within a very short time after testing began. Both 60 V and 80 V worked well, providing enough voltage to reduce the contact angle and move the droplet while not inducing almost immediate hydrolysis.

The non-conductive top plate helped reduce the contact angle between the droplet and the slide by flattening the droplet. It was able to actuate the water droplet with lower
voltages since the contact angle at zero volts was also lower.

**Fig. 11. Electrowetting 4 µL droplet on a single plate**

### 4.2 Parallel Plate Electrowetting

Parallel plate electrowetting did not work successfully, as the water droplets would not split. The electrodes used for testing were known to work, as they were used for single plate electrowetting; however, the ITO slides used for the top conductive plate did not bind effectively with the Cytop, causing hydrolysis and failure.

A few slides were tested to determine the effectiveness of parallel plate electrowetting. The first slide was tested using a 4 µL droplet at 60V and 80V. At 60 V and 80 V, elongation was induced, showing that the level of voltage applied was proportional to the amount of droplet movement. In both cases, though, the droplet was still not split. Also, at both voltages, the ITO slides failed. The Cytop was not bonded effectively to the ITO, and as it peeled off, it exposed the conductive ITO slide to the water droplets, which caused hydrolysis. None of the baking procedures tested had any effect on the ability of the Cytop to bind to the ITO. The hydrolysis not only evaporated the water droplet but also destroyed the slides. Only voltages of 40 V or below prevented hydrolysis, but they were not able to induce splitting either.

The second device used two electrode slides lying on top of each other with the electrodes aligned. On the top plate, ground was applied on the center electrode, and on the bottom slide, voltage was applied on the second and fourth electrode. This device also failed; the two normal electrode slides on top of each other did not induce hydrolysis, but they did not move the droplets either. 40 V had no effect on the droplet, and at 80 V the droplet only twitched. The area of conductivity on the top plate was too narrow to provide enough ground, so the droplet could not be split.

The devices were first tested with a 2 mm thick PDMS gasket and then switched to a thinner 500 µm gasket. The 500 µm gasket, which lowered the contact angle of the droplet, induced more movement. At 40 V, neither device induced significant droplet deformation. At 60 V, only the device with the 500 µm gasket caused any deformation. At 80 V, both devices induced deformation but the device with the 500 µm gasket had significantly more movement. Neither device was able to deform the droplets enough to split without inducing hydrolysis, but the 500 µm gasket was superior to the 2 mm one.

### 4.3 Magnetic Bead Manipulations

The 10 lb. pull strength magnet was effective in attracting the magnetic microbeads to one side of the droplet, but it was too strong and caused the beads to
magnetize. This caused clumping as the beads attracted each other, and this interfered with further movement. The 5 lb. pull strength magnet performed better, as it was strong enough to attract the magnetic microbeads, and it was weak enough to prevent the beads from congregating, allowing the microbeads to disperse again after the magnet was removed from the vicinity. This is important because in an immunoassay, the beads must have the highest surface area possible to interact with the antigens. 

While coupled with the electrowetting, however, the magnets were not successful in splitting from the droplets. The magnets were able to move the beads within the droplet, and the voltage leads were able to move the droplet, but the beads were not able to break through the solution and separate from the water.

5. Conclusions

Microfluidic immunoassays show potential as fast and efficient alternatives to traditional immunoassays. Unfortunately, the magnetic microbeads used in such models are too expensive. This experiment tested a combination of the electrowetting technique with the magnetic beads to reduce the number of beads required.

The single plate electrowetting worked well. It was able to actuate the droplets consistently, and it showed that the electrode plates worked. The procedure used is effective for both moving and merging droplets. Also, the addition of the nonconductive top plate further increased the success. Since electrowetting is able to actuate a droplet by lowering its contact angle, beginning with a flattened droplet is beneficial. This means that lower voltages can be used to produce the same effect, making it a better procedure.

The parallel plate electrowetting procedure did not work as expected. The droplet could not be split or moved. At low voltages, there was not sufficient elongation, and at high voltages, the droplet elongated but hydrolyzed as well. Hydrolysis occurs when the droplets come in direct contact with a conductive layer. The electricity causes the water to split into hydrogen and oxygen gas. The hydrolysis was caused by a poor ITO-Cytop bond. The Cytop is the protective barrier between the droplet and the conductive ITO, so when it fails, the water and the ITO can touch. This not only ruins the droplet, but it also destroys the ITO slide, and it cannot be used in further testing.

In the future, a better bonding agent such as hexamethyldisilazane, HMDS, could be used in order to improve the bonding between the Cytop and ITO so hydrolysis does not interrupt the splitting process. It was clear that the ITO slide had potential to split the droplets effectively, because at 60 V, the droplet elongated significantly. If the Cytop had not failed, the voltage could have been increased and eventually, splitting would have likely occurred.

The use of single plate electrowetting in conjunction with the magnetic bead manipulations did not work. The lowered concentration of the microbeads within the water was too low to break the surface tension of the droplet. In addition, the single plate electrowetting was incapable of splitting the droplet. In the future, once a better top slide is fabricated, parallel plate electrowetting should be combined with the magnetic bead manipulations. The parallel plate reduces the contact angle of the droplet, and the grounded top slide is able to help elongate the droplet to the point where it will split. When combined with the magnetic beads, this procedure should be able to effectively separate the beads from the rest of the fluid sample.

Another future test could use a stronger magnet to move the beads more
quickly. To counteract the effect of a very strong magnet, a surfactant could be added to the microbead solution to prevent the beads from being permanently magnetized, which causes clumping. In order to be useful in a microfluidic immunoassay, the beads need to remain dispersed to maximize the surface area exposed to the antigens.

Another way to potentially reduce the cost of the microfluidics immunoassay would be to use FTO (fluorine-doped tin oxide) instead of ITO. FTO is also a conductive substance that can be spread onto a glass slide, but it is less expensive, and this would be beneficial in making the test more economically feasible.

Although this experiment as a whole was unable to combine electrowetting and magnetic bead manipulations effectively, individual tests showed potential for future successes. Electrowetting is a conceivable supplement to current microfluidic devices that will reduce the cost of the microfluidic immunoassay and make it economically feasible.

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