NITROCELLULOSE PAPER BASED MICROFLUIDIC PLATFORM
DEVELOPMENT AND SURFACE FUNCTIONALIZATION WITH ANTI – IGE
APTAMERS

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Master of Science in Biomedical Engineering

by
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ABSTRACT

Nitrocellulose Paper Based Microfluidic Platform Development and Surface Functionalization with Anti-IgE Aptamers

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The purpose of this thesis project was to demonstrate the ability to utilize the capabilities of aptamers so that they may act as capture reagents for paper microfluidic devices. Several characterization experiments were conducted as a precursor before the final experimentation was performed. Paper characterization, manufacturing protocols for printing and heating, as well as 3D chip fabrication were all performed and analyzed. The results confirmed that the control of fluid through a 3D microfluidic device based in nitrocellulose is possible.

For the biochemistry portion of this thesis report, antibodies and aptamers were chosen to react with IgE, an antibody that is present in high concentrations in the urine of patients diagnosed with respiratory distress. Antibody chips were successfully created as a baseline lateral flow assay for comparison to new aptamer detector reagents. The aptamer experiments were able to demonstrate that it is possible to utilize the capabilities of aptamers so that they may behave as capture reagents in paper microfluidic devices. Overall, the experiments performed were extremely supportive of the ability to develop the field of paper microfluidics with the use of aptamers so that patient populations across the globe can be more accurately and effectively diagnosed.

Keywords: Paper Microfluidics, Aptamers, IgE, Diagnostics, Developing Nations

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INTRODUCTION

Specific Goals of this Thesis Project and Report

The purpose of this thesis project and report is to integrate the field of aptamers with the field of paper microfluidics. The main objective is to use aptamers as the detection reagent within a paper microfluidic chip for the purposes of identifying disease biomarkers present within patient secretions. Integrating aptamers into the paper microfluidic field would provide immense benefit to developing nations. With aptamers having such a high affinity for their corresponding analyte, but without the immune restrictions of antibodies, patients across the globe would be able to obtain accurate diagnosis in a safe, affordable, and timely manner. At the end of this process, we will have successfully shown that aptamers can be used as detection particle within paper microfluidic devices and furthermore help expand the field of diagnostics for the purposes of improving patient health across the world. We will accomplish this goal with the following:

- Characterize paper for optimum manufacturing of paper microfluidic chips
- Statistically model flow patterns in paper microfluidic chips
- Develop a three dimensional assay that will route fluid as designed
- Develop a two dimensional assay that will demonstrate surface functionalization of nitrocellulose with Anti-IgE antibodies
- Develop a two dimensional assay that will demonstrate surface functionalization of nitrocellulose with Anti-IgE aptamers

Point of Care Diagnostics: A Clinical Need

It is no question that safe, reliable, and affordable diagnostics are an emerging and prominent field in the biomedical engineering industry. This field is especially important in the developing world. Unfortunately, developing nations do not have access to the medical technology that more prosperous nations have had for decades. This means that infectious diseases that are non-existent in the United States have the ability to run rampant in foreign nations. With the health of foreign nations at stake, it is extremely important the biomedical engineering industry continues to make ground breaking developments in the field of diagnostics [1].

In developing countries, there are three diseases that cause the most devastation: HIV/AIDS, malaria, and tuberculosis. While HIV/AIDS is an epidemic in every country, it's important to note that malaria and tuberculosis are both diseases that can be treated with the means of modern medicine. The problem with the presence of these diseases in developing nations isn't the lack of technology needed to treat these diseases; the problem is simply the lack of ability to diagnose them. The impact these diseases have on developing nations is absolutely catastrophic. HIV/AIDS and Tuberculosis are much more common in the adult population than they are with children. The widespread contraction of these diseases has left countless children homeless and unable to survive without the proper care of loved ones. Diseases targeting children include lower respiratory infections and diarrheal diseases, conditions that here in the United States seem almost trivial. Some see a controversy regarding helping these developing nations with diagnostic solutions. Those opposed argue that even if diagnoses were available, many of these nations will still lack the capability of obtaining or affording a means of

treatment and possible cures. While this is a fact, it cannot be denied that the promotion of low cost diagnostic tools can help augment efforts to make treatment options more available for those who are so desperately in need [1].

Microfluidic Applications for Developing Nations

One of the main problems with diagnoses in developing nations is the inability to deploy and utilize traditional methods. In such settings, there are many complicating factors that engineers must consider when designing optimal solutions, which are not even a consideration when designing devices for the United States. Such restrictions include: low cost to manufacture and dispose, lack of trained personnel, refrigeration units, and electricity, poor laboratory equipment availability, and the likely possibility of rough handling upon transport. Given these constraints, novel Lab-on-a-Chip (LOC) devices are rapidly becoming the new standard of point of care diagnostics, especially in resource limited settings. LOCs are portable, durable, and can accurately determine the same diagnostic outcome as a well-equipped lab in a fraction of the time. Fundamentally, LOCs take in a sample, combine it with reagents, and produce output that will help determine accurate diagnoses. Some diagnostics, and in particular the diagnostic approach present in this work, can be performed in platforms that are of centimeter to millimeter length scales. Since this can be performed on a smaller scale than general lab tests, less reagents, personnel, and time is needed to perform the desired diagnoses [1].

Newly developed LOCs have the capability of using the phenomenon of capillary flow. The dynamics of capillary flow allows for simple surface tension effects to cause fluids to flow through micro and nano environments without the need of other outside energy sources, making the whole process extremely efficient and therefore reducing

cost. This makes LOCs an ideal solution for performing a diagnostic test in developing nations [2].

Samples that can be analyzed using microfluidics include virtually any human biofluid, that is - blood, urine, saliva, sputum, serum, etc. The sample being analyzed for a specific application will depend on the potential disease present within a patient. Once a sample has been chosen to optimize the possibility for biomarker identification, there are many options in terms of detection methods that can be utilized. Some microfluidic applications use immune-fluorescence detection and others use PCR methods to identify and detect pathogens. The samples used and the detection methods employed all depend heavily on the specific application that the LOC is being used for [1].

Lateral Flow Assays

Lateral flow assays are diagnostic devices that are commonly used for every day applications. Examples of lateral flow assays include pregnancy tests, ovulation tests, drug tests, etc. Lateral flow assays consist of a number of different components, specifically: the sample pad, the conjugate pad, the membrane, and the absorbent pad. The sample pad's function is to collect the patient fluids and facilitate their transfer to the conjugate pad. The conjugate pad contains the detection reagents that are used to bind and label the particles of interest within the sample (i.e. biomarkers for a specific disease state). The membrane contains the test and control lines which consist of capture reagents. The control line is used to indicate a test was performed successfully by capturing unbound detection particles. The test line indicates a positive or negative result by capturing bound biomarker/detection particle complexes. Finally, the absorbent pad is

used to collect any remaining fluid that reaches the end of the testing strip [3]. The figure below shows the components described above.

Figure 1: Lateral Flow Assay with Labeled Components

When designing a lateral flow assay it is extremely important to understand the purposes and functions of each of the components in great detail in order to capitalize upon optimum material properties. It is also important to understand how the choice of reagents and buffering solutions can affect the development of a specific assay. Once all aspects of the lateral flow assay have been understood and the application has been identified, an accurate and efficient assay can be designed and constructed.

When introducing a sample to a lateral flow assay, the first component encountered is the sample pad. The sample pad mediates the transfer of the liquid sample into the body of the device. Additionally, it provides a medium to perform sample pretreatment, that is, fluid and chemical sample modifications to ensure that the testing process can be performed accurately and efficiently. Sample fluid and chemical modifications include optimizing the viscosity, ensuring that the solution can solubilize the detector reagents, helping to prevent nonspecific binding of the sample to the test

membrane, and modifying the shape of the desired analyte to ensure that it will bind with the capture reagent. The importance of the sample pad's function can be easily explained using a simple example, i.e., a urine sample. Human urine is a common sample medium used for lateral flow assays, but it has an added complication of a varying pH (from 5 – 10). Since this scale ranges from moderately acidic to a strong base, it is important that we help standardize the pH of these samples so that we can get consistent results when performing tests on a variety of subjects. The simple addition of buffer salts to the sample pads can adjust the fluid to a desired pH that will enable all biochemical reactions to be performed optimally. Without the appropriate pretreatment in the sample pad, the other portions of the test strip will be rendered completely useless [3].

There are two common types of materials used to construct the sample pad: woven mesh and cellulose filters. Woven mesh has the ability to distribute the sample volume evenly to the conjugate pad and retain little sample volume after the transfer is complete. The drawback to using woven mesh is high cost. Cellulose fibers are thick and weak and retain much of the sample volume (when compared to the alternative woven mesh) however they are also extremely inexpensive. Overall, when choosing between woven mesh and cellulose fibers, it is also important to take into consideration the application specific thickness, dimensions and strength requirements. With all of these in mind, an appropriate sample pad may be chosen and prepared [3].

After the fluid specimen has perfused through the sample pad, it will flow through the lateral flow assay's conjugate pad. The central role of the conjugate pad is to house and introduce the detection reagents that will be used to label our analyte of interest. Once the fluid specimen hydrates the conjugate pad, the detection particles will

resolubilize, bind the target analyte and flow with the sample downstream to the detection region. The most common detection agents traditionally used are fluorescent latex beads and gold colloidal particles. In both of these cases, the label is attached to an antibody specific for the target biomarker present within the sample. Other detection reagents that have been used include enzyme conjugates, colloidal metals, dye, FITC particles, and quantum dots. The major constraints with detection reagents are particularly directed towards size and geometry. It is extremely important that the particles have a consistent and uniformly spherical shape. Larger particles move more slowly in capillary conditions than smaller particles. If the detection particles are of varying sizes, they will not arrive at the detection region at consistent times and in their intended concentrations. Instead, only a small amount of smaller particles will arrive at the detection region followed along by the remaining larger particles. This would in turn severely reduce the accuracy of the system and in turn provide possibly inaccurate results (i.e. indicating a negative result when in fact there is simply not enough detection reagent present to be detected by an observer or vision system, or a false negative). If and only if the detection particles have consistent spherical shape and size may they be considered for use in lateral flow assays. When choosing a conjugate pad, it is important that the material discourage non-specific binding, promote consistent flow onto the membrane of the lateral flow assay, and not contain particulates that may clog the pores of the membrane. [3].

Membrane selection is possibly the most important aspect of designing a lateral flow assay. The membrane is responsible for fluid routing and binding the capture reagents. Common membrane materials include nitrocellulose, Polyvinylidene fluoride,

(Charge-modified) nylon, and Polyethersulfone. Nylon and nitrocellulose both utilize electrostatic binding techniques to affix the capture reagents, while both Polyvinylidene fluoride and Polyethersulfone use hydrophobic interactions. Other important factors to consider for each specific application include membrane pore size, tensile strength, and thickness (among other characteristics) [3].

The capture reagents are placed at the test line and control line of the lateral flow assay. The capture reagents at the test line bind to the detection reagent bound to the analyte for the purposes of indicating a positive result. The capture reagents at the control line bind to the unbound detection reagents to indicate to the operator that the test was performed successfully and resulted in a sufficient concentration of detection particles being introduced to the system. Antibodies are a common capture reagent used within lateral flow assays. Since antibodies have a unique shape and can theoretically only bind to a single agent (specific binding), they make the ideal candidate for this application. In many cases, manufacturers will use an unlabeled version of the antibody that was used in the conjugate pad as a capture reagent. When using antibodies for a lateral flow assay application, it is important to verify that the antibody in question meets a few key requirements. The antibody must remain reactive after being absorbed onto a solid surface (the test line and control line in the detection area of the membrane). The antibody must retain its shape and respective structural properties when it is dried onto the test line. Finally, it must have the capabilities to be instantly active once it is rehydrated by the patient's sample. If and only if the antibody possesses these characteristics may it be considered for a lateral flow assay application [3].

Paper Microfluidics

Paper microfluidics is, in a sense, a hybrid of both classic microfluidics and lateral flow assays. Paper microfluidics relies on many of the same principals as classic microfluidics, with the exception being the material that the chip is manufactured from. While microfluidic chips are manufactured from glass, polymers, etc., paper microfluidic chips are manufactured from paper. Paper microfluidic chips are, in general, smaller, cheaper to manufacture, and of a simpler design than other microfluidic devices. Paper microfluidic chips harness the technology present in the lateral flow assay by being comprised of the same components: sample pad, conjugate pad, membrane, and absorbent pad. The main difference between the lateral flow assay and the paper microfluidic chip lies simply in their construction. In paper microfluidics, the layers are stacked on top of one another and rely on not only the lateral flow of fluids, but also on the vertical flow through the layers. Instead of being held together by plastic housing, paper microfluidic chips are constructed and held together using alternating layers of paper and double sided tape. Finally, instead of conducting a single test on a lateral flow assay, paper microfluidic chips are literally capable of performing hundreds of tests at once on a small surface. This capability relies on the designed fluid routing and the number of channels constructed for the application at hand. The World Health Organization promotes the standard that all diagnostic devices for developing countries be affordable, specific, user-friendly, rapid/robust, equipment free, and deliverable (ASSURED). Paper microfluidics is such a viable option for point of care diagnostics due to the field's ability to abide by these ASSURED specifications [4].

Nitrocellulose paper is an attractive medium for conducting diagnostic tests for a number of reasons. Paper is available in abundance for relatively cheap prices (when compared to the materials composing other microfluidic chips). Paper also has a natural wicking capability due to capillary action which completely eliminates the need for pumps or other mechanisms to drive the reactions. Paper microfluidic chips can also be easily disposed of via incineration once the testing is has finished. This completely eliminates the complication of disposal techniques and the possibility of outbreak or contamination that are possible with traditional microfluidic and diagnostic methods [4].

Quantifying the results obtained from a microfluidic device is commonly done using colorimetric techniques. Colorimetric techniques involve measuring the intensity of color and relating it to standards that will convert the intensity to a concentration. This may be done using mathematical equations and calibration curves. Colorimetric assays have been successfully developed for glucose, proteins, pH and alkaline phosphatase [4].

Other techniques for result interpretation include standard color charts that will relate color intensity to concentration. An exciting adaptation to the paper microfluidic field involves providing communication pathways between third world clinics and first world specialists. Theoretically, once a patient has allowed their sample to become fully integrated into the paper microfluidic chip, they could send a picture of the device's detection region (via camera phone, email, etc.) to a major laboratory center in another country. Technicians and specialists could then analyze the colors on the image and respond with a diagnosis and course of action for treatment. It is even possible to avoid the complications of differences in lighting conditions and screen resolutions by printing standard color charts on the microfluidic device itself during manufacturing [4]. In the

future, paper microfluidic chips could harness the potential to not only provide a diagnosis via color but also separate, purify, and perform signal communication using the provided sample via emerging technologies and breakthroughs in the field [5].

Paper Microfluidic Chip Fabrication Techniques

Manufacturing processes of paper microfluidic devices can take on multiple forms. The basic principle is that hydrophilic channels be completely surrounded by hydrophobic barriers. The fluid will then remain inside of the channels and successfully flow to the proper location for detection. There are a number of different methods that can be used to achieve this result. Martinez et al. used a technique that involved covering an entire sheet of paper with inactivated photoresist. The photoresist was activated in a selected geometry using a transparency. The inactivated photoresist was then removed using a washing technique. Aside from the impeccable geometric outcome of this method, using photoresist also allows the paper to be dried and stored for months at a time, an ideal characteristic for diagnostic devices. While this process provided the group with extremely sharp lines and accurate geometries, this technique has a few disadvantages. It isn't ideal that the hydrophilic channels be exposed to anything during manufacturing that would require a washing technique. This introduces possible contamination problems which could render the chip completely inaccurate and useless. Specifically, the process of contacting hydrophilic channels with the inactive photoresist leaves a hydrophobic residue that must be eliminated (via exposure to oxygen plasma or other cleansing method) [4].

Another method used to obtain the required geometry utilizes printing techniques. First, a wax printer is used to generate a 2D pattern of the required geometry on the

surface of the paper layer. Second, heating techniques (hotplates, oven, etc.) are used to allow the wax to penetrate the entire thickness of the layer. The disadvantage of this technique lies in the accuracy. It is simply impossible to obtain resolution produced by photoresist with printing and melting techniques. However, regardless of the lack of high resolution, printing techniques provide many key advantages. Printing the geometry takes significantly less time than using photoresist techniques, the test zones and channels are never exposed to any chemicals that may contaminate them, there is no drying involved, and different colors can be easily incorporated into the design (if needed). If desired, the sheets can even be easily loaded back into the printer to allow for the deposition of reagents to the chip's test zones. This would eliminate the need to deposit reagents using more complicated measures (micropipettes, strippers, etc.) [4].

Shaping, or cutting, is another method that can be employed in order to manufacture paper microfluidic chips. The process of cutting includes the shaping of porous media (cellulose paper, nitrocellulose paper, etc.) using a computer controlled cutting tool, usually a knife. The advantages of using this sort of technique are that it is a suitable way to produce paper microfluidic chips in rural areas, it can produce a high volume of chips in a small amount of time, and it also can significantly reduce the amount of operator error by controlling most of the process with computers [6].

Fenton et al. used a computer controlled x – y knife plotter to create a wide variety of geometries on the faces of paper microfluidic chips. This machine is just like a standard plotter, but with a knife to replace the pen. The knife is freely rotating, allowing the team to make precise cuts to produce intricate geometries. They used the plotter to create the geometry of the channels and claim that the entire process takes less than a

minute to complete. After the knife has finished cutting, they must unload the paper and then manually cut away the extra material. The team recommends the addition of plastic films to help eliminate contamination as well as keep the system from losing any of the liquid samples to evaporation. This thinner alternative to covering the chip will is also a great alternative from using the bulky plastic housing that is currently common practice [6].

Plasma treatment fabrication is another method that could be considered for the purposes of manufacturing paper microfluidic chips. Recently, Li et al. created a plasma treated microfluidic chip that was able to successfully perform using traditional colorimetric methods. They first took filter paper and made it hydrophobic using AKD – heptane solution. In order to create hydrophilic channels, the team first manufactured stainless steel stencils and placed them on either side of the hydrophobic paper. This stack of stencils and paper was placed within a chamber and exposed to a vacuum plasma treatment. After the treatment had been applied, the group noted that, visually, no alternations had been made to the sample due to the exposure [7].

The group was able to verify their design by conducting a color changing enzyme reaction on the surface of the chip using alkaline phosphatase. They noted the expected color changes and deemed their manufacturing a success. The group claims that their technique for fabricating paper microfluidic chips has some extreme advantages. First, it is important to note that the areas that are not exposed to the plasma treatment remain completely hydrophobic. As mentioned before, there is future potential for more advanced processes to be performed on the surface of paper microfluidic chips. For example, switches and separators could be incorporated into the hydrophobic areas and

allow the samples to undergo more in depth analysis. Switches could be incorporated in order to control flow capabilities, specifically to turn the system “on” and allow it to flow, or turn the system “off” and cause it to stop. Separators also have a special function. In classic microfluidics, separation is a common technique used to separate raw samples into particles of interest and particles of disinterest. Separators that have been incorporated into paper microfluidics would allow for more in depth analysis on samples that are of more pure quality. This would in turn produce more accurate results [7].

Fluid Flow Characteristics in Paper Microfluidic Chips

Fluids are able to flow throughout the pores and channels of paper microfluidic chips due to both the wicking properties of paper as well as the physics of capillary flow. The movement of the fluid can be easily described using the dimensionless parameter simply known as the Péclet number. The Péclet number is a dimensionless quantity that compares a particle’s transport by fluid flow to transport by diffusion. The Péclet number is also known as the product of a fluid’s Reynolds number (the ratio of internal forces to viscous forces of a fluid) and the Schmidt number (the ratio of momentum diffusivity to mass diffusivity). By using the properties such a Péclet number, we can see that the fluids flowing through a paper microfluidic chip will be exposed to both convective transport as well as diffusive effects.

Classic microfluidic chips are able to incorporate mechanisms that allow them to control the flow rate of a sample, manipulate target particulates in fluids, and control the mixing time. In order for paper microfluidics to achieve its full potential as a reliable method for point of care diagnostics, it will eventually need to incorporate these capabilities as well. Controlling the flow rate of a sample through a paper microfluidic

chip would be the first step towards working to the other mechanism goals mentioned above. Multiple factors play a role in determining the wicking speed of a sample within the channels of a paper microfluidic chip. These factors include: dimension of the chip's channels, the fluid viscosity, as well as the temperature and humidity of the testing site [8].

Ideally, assays present on a paper microfluidic chip should develop simultaneously, eliminating the need for a user to wait around for multiple results. Seeing as you can potentially put hundreds of assays and detection regions on a single 3D paper microfluidic chip, the development time between assays could sum to an unreasonable period of time. Thus, controlling the wicking rates of the individual assays is a key design consideration. There are multiple ways that can be used to control the flow rate within a paper microfluidic chip. The first and most common method is to modify the channel width. Addition of wider channels would effectively increase the amount of time the fluid would need to travel from the input region to the detection region. However, wider channels will require more sample volume. They will also significantly increase the total size of the paper microfluidic chip and result in a more expensive overall product. As a consequence, increasing the required sample volume could exceed realistic conditions. For example, if the fluid being analyzed was tears, getting a larger sample could be extremely difficult, whereas if the sample fluid were urine, it would make less of an impact [8].

A second method of fluid rate control involves modulating the wetting properties of the aqueous solvent. Noh et al. [8] recently focused their efforts towards this endeavor. In order to modulate the wicking properties of water, they selectively

impregnated the microfluidic channels with small droplets paraffin wax (these were referred to in the paper as “meters”). Hypothetically, the larger the volume of wax, the slower the aqueous solvent would wick through that particular channel. To illustrate this phenomenon, the team used food coloring as a means of testing the microfluidic chips. At the start of each test they filled the input location completely with their colored sample and the assay was considered complete when the food coloring was present within the entire detection region. The paper layers were manufactured using the laser jet printing technique that has been described previously in this introduction. The double sided tape used to connect the layers of paper was manufactured using a laser cutting machine. The meters were delivered to layers of paper using a hand delivery technique. It is possible that in future iterations of this technique that the wax meters may be able to be delivered to the paper using the same ink jet printing technique that produces the channel boundaries. Overall, the tests performed by this group allowed for the conclusion that there was a non-linear increase in development time when more meters were added to the chip. This experiment successfully showed that the development time of aqueous solutions through a paper microfluidic chip can be engineered to produce specific results [8].

A New Detection Reagent: Aptamers

While making headway in terms of the manufacturing and metering of paper microfluidic devices are important and necessary developments, it is also important to continue to make strides in terms of revolutionizing the biochemical components of paper chips. Classically, antibodies have been used for years as capture agents for human biomarkers. They have high specificity, and up until now have been the standard for

specific selection in biochemical fields. However, there is a new class of detector particles that can be used for paper microfluidic applications with as much if not more success as the current antibody standard. These particles are known as aptamers.

Aptamers are simply oligonucleotides (DNA or RNA) that have the capability to bind to specific target molecules. Target molecules contain multiple classes including drugs, proteins, organic molecules, inorganic molecules, etc. The function of aptamers is the same as that for antibodies. Aptamers have the ability to target specific molecules based on shape. While the functions are the same for aptamers and antibodies, the use of aptamers has some extreme advantages. Antibodies begin to denature at 40 degrees Celsius, while DNA and RNA strands will begin to denature at 90 degrees Celsius. The ability of aptamers to be able to withstand extreme environments makes them an ideal candidate for use in developing nations. While antibodies must be generated by a live host in vivo, aptamers are generated using in vitro conditions. When harvesting antibodies, scientists and engineers must stimulate an immune response within a host in order to generate the desired antibody. Common problems concerning this method include confusing the desired antibody with others that look and behave similarly as well as the potential toxicity of the antigen. Since antibodies are produced by a living host, the immune system determines the shape, binding capabilities, and binding sites of each antibody. With aptamers, these properties can be engineered based on the application at hand. Scientists and engineers can literally control the binding location, the bond type, and the binding conditions of each specific aptamer. Finally, aptamers can also be modified and purified, which enhances their affinity to bind to their specified target [9].

Aptamers are generated using a procedure known as SELEX. The first step in this process includes generating a large single stranded library of oligonucleotides. The classic Polymerase Chain Reaction, or PCR, is used to duplicate each oligonucleotide so that it is in detectable concentrations. The library is introduced to the molecule of interest (i.e. the molecule that is to be detected by the new aptamer, also known as the biomarker). The oligonucleotides are allowed to interact with this molecule until some sort of attachment has been made [9].

Next, a filtration step is performed to remove all oligonucleotides that have not adhered to the molecule of interest. This filtration can either be in the form of a positive or negative selection technique. Positive selection techniques consist of filtering out all potential aptamers that fail to specifically bind to a molecule of interest. Negative selection techniques are also another way to select for potential aptamers. Negative selection includes filtering the potential aptamers through cellulose fibers. Any particles that form non-specific attachments with the fibers will be filtered and eliminated from the selection process. Once either a positive or a negative selection step has been performed, another amplification step is performed to increase the concentration of the potential aptamers. This step is PCR for all DNA molecules and RT PCR for all RNA molecules. This cycle of amplification and filtration is continued for much iteration until a purified sample of the aptamer for the specific molecule has been obtained. This entire process of aptamer development can take weeks or even up to months in time to complete. Because of this huge time commitment, the entire process has been automated, allowing engineers and scientists to carry on with other experimentation while the aptamer selection process is occurring [9].

There are many applications for the use of aptamers. These include things such as chromatography, optical sensors, biosensors, etc [9]. Recently, Nguyen et al. used aptamers to enrich samples used for microfluidic applications. Their microfluidic chip contained a chamber with aptamers that attached to, enriched, and released target molecules. First, the aptamers were introduced to the system so that they may bind to their target molecules. This step was followed by a “flushing” step to remove all impurities. The aptamers were then chemically signaled to release their target molecules so that further microfluidic analysis may take place. Using aptamers to purify samples is an extremely useful way to integrate aptamers into microfluidics. When running a sample through a microfluidic chip, much of the sample itself is useless. It is simply surrounding material that flows through the channels with the molecules of interest. Since aptamers have such a high specificity for their target molecules, it is safe to say that purifying samples with aptamers before running microfluidic analysis will severely decrease the required sample size, which will save time, material, and money[10].

Project Specific Aptamer Selection: Anti – IgE

When it comes to selection of an aptamer to test for this thesis project, it is important to consider an application that will be applicable to the developing world. Chronic respiratory diseases are a growing problem in developing nations mainly due to their extremely high frequency and their economic impact on the country itself [11]. Bronchial asthma is a growing problem in developing nations due to the use of biofuels and poor ventilation systems. Bronchial asthma is characterized by inflammation of the lower respiratory tract, bronchial hypersensitivity, and reversible airway obstruction [12].

Approximately three billion people worldwide rely solely on biomass (burning of wood, charcoal, crop residues, and dung) and coal for energy in their personal homes. The burning of biomass accounts for about half of the energy used in domestic nations. Byproducts from using biomass fuels include carbon monoxide, nitrogen dioxide, formaldehyde, etc. Diseases caused by inhaling these byproducts include asthma as well as chronic obstructive pulmonary disease (COPD), cancer, and tuberculosis among others. The National Ambient Air Quality Standards set forth by the United States Environmental Protection Agency mandates that the average daily concentration of particulate matter that anyone should be exposed to should be less than 150 ug/m^3 (where particulate matter is defined as any airborne particle that is less than 10 micrometers in diameter). Homes using biofuels for energy in developing nations can expect to have particulate matter concentrations range from $200 - 5000 \text{ ug/m}^3$, significantly above the amount that is recommended [13].

In 1967 it was discovered that Immunoglobulin E (IgE) is responsible for the allergic reactions experienced by patients with allergic diseases through the stimulation and attachment of mast cells and basophils. When compared to the other Immunoglobulin proteins present within the human body, IgE is by far present in the smallest amounts within human serum. Higher levels of IgE are indicative of allergic diseases (i.e. the higher a patient's serum IgE levels, the more severe allergic disease they are inflicted with). This characteristic of IgE makes it ideal for use in diagnostic devices. [14].

Traditionally, Enzyme Linked Immunosorbent Assays (ELISA) are used to detect the presence of antibodies (like IgE) present within patient samples. ELISA kits are used

to determine the presence of antibodies both qualitatively and quantitatively. ELISA kits utilize the capabilities for antibodies to have high specificity for their respective antigens in order to make the detection process accurate and efficient. ELISA kits work by first tethering a reactant (either an antibody or an antigen) to a solid supporting surface. In most cases, this solid surface comes in the form of a 96 plastic well plate which can be used for 96 separate ELISA tests. The reason that plastics are often used in ELISA kits as the solid surface is due to the fact that plastics have the capabilities of forming monolayers of proteins without denaturing them and rendering them incapable of binding to their corresponding antigen [15].

After the antibodies have been bound to the plastic wells, a blocking agent will be applied to the exposed portions of plastic that are not attached to an antibody. This blocking agent is usually Bovine Serum Albumin (BSA). BSA will inhibit unbound sections of plastic from attracting antigens or other particles and forming non – specific bonds that will ultimately skew the results of an ELISA test (indicate a result is positive when it is really negative). Once the plastic membrane has been completely treated, a test solution potentially containing the particles to be detected is added to the wells. If present, the antigens within the solution will bind to the tethered antibodies attached to the plastic wells. Since the bound particles will be securely attached to the wall of the wells, it will be easy to determine and separate the bound particles from the unbound. As a final step, an enzyme conjugated reagent will be added to the wells for the purposes of amplifying the bound particles and a color reagent will be added so that the bound particles can be visualized [15].

It has been found from multiple studies that the affinity for the IgE aptamer has a much higher affinity for IgE than anti – IgE giving the advantage to aptamers over antibodies. Also, as stated before, aptamers have a higher resistance to degradation when exposed to extreme conditions (temperatures, pH, humidity, etc.) than that of antibodies, which makes them a much more attractive choice for use in diagnostic devices for developing nations [14]. Aptamers also have the advantage of containing binding sites that can be easily manipulated, they can be modified with functional groups for covalent tethering to multiple surfaces, and they can be created for virtually any target [16].

IgE aptamers have already gained recognition and have been used already for multiple diagnostic devices. Yun Hwa Kim et. al successfully was able to produce an aptamer biosensor for the purposes of detecting IgE in patients with allergic diseases using the capabilities of surface plasmon resonance (SPR) [14]. Karin Stadtherr et. al was also able to create a functional protein biochip using IgE aptamers [16].

To use IgE aptamers as the attachment particle for a paper microfluidic device for the developing world would be a great achievement for the field of patient diagnostics. It would eliminate the multiple step processes required by traditional ELISA testing, and could theoretically produce more specified detection through the use of new reagents. An IgE aptamer paper chip would help pave the way for utilizing the capabilities of aptamers in up and coming paper devices, as well as provide a platform to aid in the diagnosis of chronic respiratory diseases in developing nations.

Organization of the Remainder of the Report

The remainder of the paper is organized as follows. In the methods section, the protocols for successfully designing and manufacturing 3D colorimetric nitrocellulose

paper chips and 2D IgE antibody and aptamer chips will be explained in detail. Next, the results of the multiple attempts to develop the protocols will be presented. Finally, the implications of the results as well as recommendations for future work will be discussed.

MATERIALS AND METHODS

A number of experiments will be designed and conducted in order to fully analyze and quantify the capabilities of the microfluidic chip. First, 2D designs will be created and used to characterize the basic properties of our chip design and paper capabilities. Standard colorimetric techniques will be employed in order to determine parameters such as wicking time, line thickness requirements (so as to not allow the fluid to leak outside of the testing channels), etc. Once the characterization experiments have been completed and the properties of our paper and geometries are well understood, work will be done on a more complex three dimensional design. Developing the 3D assay will involve the standard colorimetric techniques. These experiments will be done in order to ensure that the 3D design created is able to wick the fluid up through its individual layers as designed. The 3D chip will also be evaluated on its ability to produce results in the detection region in a specific order, designated by the specific design of the chip. The ability to perform this experiment correctly will demonstrate the ability to control the flow of fluids through a paper microfluidic chip.

The second set of experiments will involve detection capture reagents that would be used in an actual device. These experiments will involve a more complex scheme than simply colored fluid. It will require the target IgE analyte to bind with an Anti-IgE FITC detection particle and only as a result of this binding will we be able to determine a positive or negative result. Experiments at this stage will allow us to characterize our testing capabilities and our ability to create a chip that will detect specific particulates within a given sample. First, a 2D chip will be created and impregnated with reagents for an antibody assay. This will include an IgE target, an Anti-IgE FITC detection reagent,

and an Anti-IgE capture reagent. Once we have successfully developed the antibody experiments, we will use what we have learned and apply it to the less studied IgE aptamer capture agents. Methods of imaging the detection aptamers at the detection region will prove the most crucial part of this project. Once all experiments have been completed with satisfactory results, this thesis project will be considered a success.

Paper Characterization Experiments: 2D Paper Microfluidic Devices

Characterization of Development Time

The first phase of this thesis assignment consisted of creating and performing experiments for the purposes of characterizing the properties of various types of paper as well as discovering relationships between the geometric measurements of the channels and how they can relate to development time (the time it takes for fluid to wick through a specified area of paper).

The first experiment that was performed was used to generate a statistical model of characteristics that will affect the fluid development time for a paper microfluidic chip. In order for the analysis to be carried out, chips containing single vertical channels with varying lengths and widths were designed using the AutoCAD software. The file containing the chips with varying thicknesses and lengths is shown in the Figure below.

Figure 2: Image of AutoCAD file of chips that were used to create the development time model.

The chips were printed using a Color Qube 8570 wax printer from Xerox on standard bulk printing paper. The printer is shown in the figure below.

Figure 3: Color Qube 8570

The chips were heated in a Binder FD series heating oven set at 150 degrees Celsius for 30 seconds. The oven used for the experiments is shown in the figure below.

Figure 4: Binder FD Series Oven

In order to generate a statistically sound model, many variables were considered and determined experimentally. Development time was determined by applying water containing food dye to the end of the channel contacting the horizontal “reservoir”. Once the fluid had been applied, time was started. Once the fluid had filled the entire channel, the time stopped and the total time elapsed was recorded as the variable “development time”. As stated before, the paper chips were designed with varying channel widths. AutoCAD was used to accurately determine channel thickness. After printing, the chip was heated to allow for the wax to penetrate the entire thickness of the paper. Since this heating also caused the wax to spread horizontally and could potentially change the specified value of the channel width, the width of the channel was measured using a ruler in order to detect any possible changes. The actual width that was measured was recorded as the variable “width”. As stated before, paper chips were designed with varying channel lengths. AutoCAD was used to accurately determine channel length. After printing, the chip was heated to allow for the wax to penetrate the entire thickness of the paper. Since this heating will also cause the wax to spread horizontally and could potentially change the specified value of the channel length, the length of the channel was

measured using a ruler in order to detect any possible changes. The actual length that was measured will be recorded under the variable “length”. Since the paper used for this experiment was bulk copy paper there is a strong possibility that different sheets of paper will have varying pore densities and diameters. To account for these potential discrepancies, blocking by paper will be considered when performing the statistical analysis. Paper blocking will be achieved by performing each test with each chip twice using two separate pieces of paper. The sheet of paper that was used for each experiment was recorded under the variable “paper”.

In order to generate the appropriate model that was needed to accurately and effectively model the data, statistical transformations were needed in order to ensure that the residuals were normalized and that the variance was homogeneous. Some of the data points were extreme outliers that significantly skewed the data. Once these points were identified, they were removed from the analysis to allow for a more accurate model to be generated. After appropriate transformations had been determined and performed and the outliers had been removed, a general regression was performed using the capabilities of Minitab in order to compute the appropriate models. For a more detailed account of the modeling process, please see Appendix B: Detailed Statistical Analysis.

Paper Characterization

The second goal of the 2D experiments was to determine specific paper characteristics. This experiment was completely qualitative. Three forms of paper were used. The first was bibulous paper purchased from the local bookstore for use in microbiology and chemistry laboratories. The second was an unbacked nitrocellulose paper. The third was a backed nitrocellulose paper from Millipore (HF13504XSS). For

the first two paper types, three arbitrary geometries were created in AutoCAD and printed onto each of the three paper types. The geometries had 1, 2, and 3 channels respectively. After printing, each sheet was heated in the oven for 30 seconds at 150 degree Celsius. Once cooled, water containing food coloring was deposited onto the corner of each chip and allowed to wick through the channels. The backed nitrocellulose from Millipore was used to create the same geometries as before, but with three different channel widths. These channel widths were 0.8mm, 1.6mm and 2.4mm. An image of the file containing these geometries is shown below.

Figure 5: Paper characterization with varying channel widths

Construction and running of the experiment was performed the same as with the other paper types. Observations regarding the ability for the fluid to wick through the channels of each of the three paper types were noted and recorded in a laboratory notebook.

Colorimetric Experiments: 3D Paper Microfluidic Devices

It is important to consider all options when choosing a fabrication technique for producing paper microfluidic chips. Paper microfluidic chips have been successfully

constructed using a variety of methods including photolithography, plotting, plasma oxidation, cutting, and inkjet printing [17]. For the purposes of this thesis report, we will be manufacturing the microfluidic chips using the inkjet printing technique and achieve stacking and attachment of the individual layers using double sided tape that has been shaped using a laser cutter. When compared to all other methods, inkjet printing is the most rapid and simplest method to produce mass amounts of chips. For the purposes of research, it is important that we be able to manufacture our prototypes quickly and efficiently with less emphasis on extreme accuracy. Bearing all of this information in mind, it made sense to go forth and utilize the inkjet printing technique.

Inkjet printing comes with some drawbacks. Paper is anisotropic, meaning that when heated, the ink will penetrate in the horizontal direction (across the surface) faster than in the vertical direction (throughout the paper's thickness). When the chips are first printed, they will be produced exactly as they are specified in the drawing file (with consideration to the limits of the resolution of the printer). This is because the wax will instantly cool and solidify once it leaves the printing cartridge and touches the paper. There are a variety of heating techniques that can be used for the purposes of fabricating paper microfluidic chips including hot plates, ovens, and heat guns [17]. For the purposes of this thesis, and given the tools at hand, we will be utilizing the capabilities of an oven for this project.

Even given these drawbacks, the method of printing and heating will still be used for the purposes of this thesis report. For the purposes of this report, we will be looking more for general attachment and expression of biomarkers. While the geometry of the

chip has some importance, the discrepancies introduced by this method will not provide any aspects that would prove to be a detriment to the success of the project.

The following design was developed in AutoCAD for the purposes of constructing and testing a 3D colorimetric paper microfluidic chip.

Figure 6: 3D Microfluidic Chip Layers

The layers are designed to be assembled (from the bottom to the top) in the order from top left to bottom right. The white layers represent layers of paper with wax (the black regions are fluid routing channels). The top left white layer is backed; all other white layers are unbacked. The yellow layers represent tape layers. The black regions represent laser cut holes for fluid routing. The red circular shapes represent conjugate pads. The red rectangular shapes represent absorbent pads. The bottom right layer represents a transparency that will be constructed for viewing purposes as well as protection of the assay from contaminants. This chip was created in both a larger and smaller scale for the purposes of testing. The smaller chip was created to optimize the flow properties using methods that were determined in previous experiments. The larger

chip was created to display the tradeoff with less than desirable flow characteristics for optimal visual representation of the final detection area. For specific dimensions, please see Appendix C: Drawing Files and Dimensions.

Printing Methods

Multiple printing methods were used in order to determine the most accurate and reproducible. The method used for backed membranes is simple. For this experiment, the backed membrane that was used was Millipore HF13504XSS. The paper was cut using titanium scissors to the size of a standard piece of copy paper. It is important to use titanium scissors, as other scissors will tear the nitrocellulose. The scissors used for this experiment and all experiments following are shown in the following figure.

Figure 7: Titanium Scissors

Once the paper was cut to size, it was placed into the paper tray of the printer and printed like any other normal sheet. Once the heating step has been initiated, the anisotropic properties of the paper will come into play. The properties of the paper will manifest as a couple of characteristics present within the fully manufactured chip. First, there will be a significant difference in line thickness between how the chip was drawn in AutoCAD (how it is printed) and how it will be presented once the chip has been heated. The lines in general will appear thicker than they did originally, which will decrease the general resolution of the chip itself. Also, since there is a difference in the diffusivity rates in

both the horizontal and vertical direction, the lines on the bottom of the chip will appear thinner than the lines on the top [17].

There were multiple methods for printing on the unbacked membrane that were tested for reproducibility and efficacy. For this experiment, the unbacked membrane that was used was Millipore HF13504XSS. The first method that was attempted was to print using the same protocol as for the unbacked (as mentioned before). The second was to develop a paper template made out of backed nitrocellulose that contained the printed layers on the corners of the sheet. An example of such a template is shown below.

Figure 8: Template used for printing unbacked nitrocellulose

Unbacked nitrocellulose was then cut into smaller squares and placed on top of the template where the printing would occur. In this method, there was nothing physically attaching the unbacked to the backed nitrocellulose. The electrostatic interactions between the paper layers were relied on to keep them together. This template with the unbacked paper was fed into the printer and was printed like before. The third method was to create the same template as before, but on regular copy paper. Unbacked nitrocellulose was cut into small squares and placed on top of the template where printing would occur and then held in place using scotch tape. This sheet was then placed into the

paper tray and printed like before. Qualitative results regarding reproducibility and reliability were recorded in a laboratory notebook.

Unbacked Paper Layer Printing Protocol

The specific results regarding each of the methods attempted will be discussed later in this paper. The following is a specific protocol outlining the steps for the most successful method for creating the unbacked layers: the third template/taping method.

In AutoCAD, display all of the unbacked layers onto one drawing. Space the layers out so that they will fit approximately on the corners of a standard sheet of paper. Print the template onto standard copy paper to ensure proper placement. The file that was used for this project is shown below.

Figure 9: AutoCAD file with paper corner alignment

Print this file onto a plain blank sheet of paper. Make sure you have selected the appropriate window, centered the plot, selected the correct paper size, and entered in your appropriate unit scale into the dialog box. An example of this “template” is shown below.

Figure 10: Paper Template

Flip this page over on the benchtop so that the wax portion is facing down. This step is shown in the figure below.

Figure 11: Paper Template Ready for Assembly

Next, cut out sections of unbacked nitrocellulose paper that are large enough to cover the parts of the paper that have been printed with wax while still allowing for tape to cover the perimeter. Place these pieces onto the template and tape them into place using scotch tape. This step is shown in the figure below.

Figure 12: Placing the first unbacked square

Once this square has been placed, the sheet should look like the following figure.

Figure 13: Template with first unbacked square placed

Continue this process until all four corners have been completed. Then place this template into the paper tray and print the document as before. Be sure not to change any of the settings in the dialog box to ensure that the document is printed exactly the way it was when creating the template. The document should come out looking like the following figure.

Figure 14: Successfully printed unbacked nitrocellulose

Next, use the titanium scissors to cut the unbacked nitrocellulose from the paper template. This step is shown in the figure below.

Figure 15: Cutting the unbacked layer from the paper template

Once you have cut all of the layers from the template, the process of printing unbacked layers is complete.

Melting Protocol

After paper layers have been manufactured, the next step is to melt the wax to allow it to penetrate the entire thickness of the paper. This was done by heating all layers to 125 degrees Celsius for thirty seconds in an oven [18]. It helps to place the layers onto a larger sheet of printer paper so that they do not accidentally fall in between the rings of the oven sheets. The figure below shows this step.

Figure 16: Printing the paper layers

Tape Layer Manufacturing

Double sided tape from 3M was used for this thesis project. To prepare for laser cutting, the tape was unrolled and laid out onto a sheet Reynolds Parchment Paper. This step is shown in the following figure.

Figure 17: Backing the tape with parchment paper

Titanium scissors were used to cut off a desired length of tape. This step is shown in the following figure.

Figure 18: Cutting tape to desired length

Next, the wax paper was smoothed by hand to try and remove air bubbles that had become trapped during the rolling process. This step is shown in the figure below.

Figure 19: Smoothing parchment paper and removing air bubbles

Then the excess parchment paper was cut away from the tape's perimeter. This step is shown in the figure below.

Figure 20: Cutting away extra parchment paper

This tape/parchment paper was then laser cut using a Versa Laser Cutter. A picture of the laser cutter is shown in the figure below.

Figure 21: Laser Cutter

Before cutting tape layers with a laser cutter, create an AutoCAD document containing multiple copies of your tape layers. This way, you can create multiple copies of each layer in one run through. It is important that this AutoCAD file have red lines of thickness 0.00 and be saved as a .DWG 2007 file. This particular laser cutter reads by color, and will only make cuts on red lines. The 0.00 thickness is to ensure that the laser cutter only makes one pass on each line. If the line thickness is anything but zero, the laser cutter will make multiple passes, which is unnecessary. The file that was used for this experiment is shown in the figure below.

Figure 22: Tape Layer File

Load this file onto the laser cutter computer and open it. Create two layers for this file, one that contains all details within the tape layer, and another that contains all of the perimeter lines. This will make the printing process much easier. Turn off the layer containing the perimeter of the tape layers. Print the file, and select the laser cutter as the printing destination. Make sure that the scaling is right, the correct window is selected, and that the plot is *not* centered. Your drawing should appear at the bottom left hand corner of the printing surface shown in the dialog box. Click ok.

Open the laser cutter program interface. Click settings and change the material properties to 0.5mm Mylar for cutting the tape [19]. Place the tape/parchment paper into the laser cutter so that it aligns with the bottom left hand corner of the platform. Secure the tape in place by taping it to the platform using masking tape. This step is shown in the following figure.

Figure 23: Placing the tape into the laser cutter

Close the lid, check to make sure that the exhaust is on, and click play. Wait for the laser cutter to finish. Check to see that the laser cutter cut through the entire thickness of the tape. If it didn't, close the lid and click play again. Wait for the laser cutter to finish.

The tape layers should look something like the following figure.

Figure 24: Inner details of tape layers cut away

When this is done, repeat all steps again but this time with the inner details layer turned off and the perimeter layer turned on. When finished, turn off the laser cutter and shut down the computer. The final product should look something like the following figure.

Figure 25: Completed tape layers

Creating a Bridging Layer Between Paper Layers

In order for the fluid to flow through the entire width of a chip, there needs to be a bridging layer that passes through the holes of the tape and contacts the nitrocellulose paper on both sides. There were multiple methods that were tested when optimizing a bridging layer. First, a bridging layer was created by crushing nitrocellulose paper dissolved in acetone with a mortar and pestle. This paste was then dried overnight before

use. Second, a bridging layer was created by cutting nitrocellulose with scissors into shapes that would fit precisely within the holes of the tape. Finally, a bridging layer was created by crushing nitrocellulose paper dissolved in water with a mortar and pestle. This paste was placed onto bibulous paper to remove excess water before being placed immediately on a chip being assembled.

Bridging Layer Manufacturing Protocol

The specific results regarding each of the methods attempted will be discussed later in this paper. The following is a specific protocol outlining the steps for the most successful method for creating a bridging layer: the “fresh paste” water method.

First, take some nitrocellulose and tear it into small pieces. Place these small pieces into a mortar. Fill the mortar with a bit of water (this is not an exact science; enough to soak the paper is fine). A picture of this step is shown in the figure below.

Figure 26: Water and Nitrocellulose, ready to be crushed

Crush the paper in the water until it has partially dissolved and looks like the figure below.

Figure 27: Bridging layer after crushing

Pour out this mixture onto bibulous paper so that the extra water can be removed from the nitrocellulose. Set aside this “paste” to be used during assembly. It should look like the following figure.

Figure 28: Bridging layer

Pad Manufacturing

Finally, the conjugate and absorbent pad will need to be manufactured. Both pads were created using the Millipore glass fiber conjugate pad strips. To create an absorbent

pad, cut the strips so that sections are approximately $\frac{1}{4}$ " thick. The absorbent pads should look similar to the figure below.

Figure 29: Absorbent Pads

In order to create a conjugate pad, cut a longer section of the strip and place it over a weigh boat as shown below.

Figure 30: Preparing the conjugate pad

Slowly drop food coloring onto the conjugate pad until the entire strip has absorbed color. This step is shown in the figure below.

Figure 31: Adding color to the conjugate pad

Place the soaked conjugate pad onto a piece of bibulous paper so that all excess color can be drained. Allow the pad to sit until it becomes completely dry. This step is shown in the figure below.

Figure 32: Drying the conjugate pad

Finally, cut this strip into pads that are approximately the same size as the absorbent pad. This step is shown in the figure below.

Figure 33: Finished conjugate pads

Chip Assembly

The following figure shows all layers that will be assembled into one final chip.

To begin the chip assembly, begin with the backed paper layer on a flat surface. Remove the wax paper from the first tape layer and place it, sticky side down, onto the backed

layer. Make sure that the holes in the tape line up with the circles on the paper layer.
The assembly should look like the figure below.

Figure 34: Backed layer with first tape layer

Remove the other side of the tape backing. Add bridging layers to each tape hole. After this step, the chip should look like the figure below.

Figure 35: First bridging layer

Next, add the first unbacked layer to the top of the assembly. Make sure to try and line up the circles on the paper layer to the bridging layer. The assembly should now look like the figure below.

Figure 36: First unbacked layer

Next, place the conjugate pads onto the paper layer ensuring that they span the entire width of the wax “spacer” that separates the two channels. The purpose of the spacer is to ensure that fluid will travel up through the conjugate pad and not simply pass underneath it. The assembly should now look like the figure below.

Figure 37: Conjugate pad placement

Next, remove the wax paper from the second tape layer. Place it on top of the assembly making sure to not move the conjugate pads, as well as ensuring that the holes of the tape line up with the circles on the paper layer. The assembly should look like the figure below.

Figure 38: Second tape layer

Remove the backing of the tape and add bridging layer to the holes as before. The assembly will now look like the figure below.

Figure 39: Second bridging layer

Add the final unbacked paper layer to the assembly. Make sure to try and line up the circles on the paper layer to the bridging layer. The assembly should now look like the figure below.

Figure 40: Final unbacked paper layer

Place the absorbent pads on top of the left most circle on this final layer before removing the wax from and placing the final tape layer. The assembly should now look like the figure below.

Figure 41: Final tape layer application

Finally, remove the backing of the final tape layer and place a piece of transparency on top of the assembly. The chip is now finished and should look like the figure below.

Figure 42: Finished chips

Performing the Colorimetric Experiment

Place water onto the sample region of the chip (the square on the bottom left hand corner). Wait for water to flow through the entire chip, replacing water on the sample region as necessary. If the chip performs successfully, colored water should appear and travel through the entire top region.

Antibody Experiments: 2D Paper Microfluidic Devices

For the purposes of creating and testing an antibody chip, the following AutoCAD file was created containing multiple chips.

Figure 43: Antibody chip design

For dimensions of this file, please see Appendix C: Drawing Files and Dimensions. The chips were printed and heated in the same procedure as previously described for backed layers.

Biochemistry for Antibody Chips

The solution containing the capture reagents must be buffered for optimization of specific characteristics. The main purpose of buffering the reagent mixture is to control the pH of the solution. In order to limit the ability of the buffer to denature the antibodies being used, it is important that the smallest concentration of buffer required to meet the specification be used for the application. At a minimum, the buffer should be reduced to a concentration less than 10mM. When selecting a buffer, Millipore recommends the elimination of salts (including NaCl), surfactants and detergents including Tween 20 and Triton X – 100, as well as any additives. Alcohols, specifically methanol, ethanol, or isopropanol may be used within this buffer solution to lower the solutions viscosity, surface tension, and static repulsion. Each of these chemical modifications will in turn improve the consistency of the application of the reagents to the test line. The presence

of alcohol in the reagent solution will also decrease the solubility of proteins without the risk of denaturation and in turn help them dry and fixate onto the membrane's test line. Finally, the addition of detergents to the test line helps to rewet the area to help prevent deformed flow. Deformed flow, backflow and uneven wicking can cause serious discrepancies in the results. Backflow especially causes possible contamination and false results. Therefore, it is important when designing and manufacturing a test line that detergents be used to help prevent this from happening [3].

In order to prepare buffering solutions for the experiment, the following steps were followed to create stock solutions for use later in the experiment. For all calculation information that was used to come up with the following methods list, please see Appendix D: Biochemistry Calculations.

To prepare ammonium acetate stock solution

- Measure out 0.002 g of ammonium acetate onto a scale
- Add the measured ammonium acetate to 10mL of water
- This combination will produce a 0.00260 molar ammonium acetate solution

To prepare IPA stock solution

- Measure out .100mL of IPA
- Add the measured IPA to 5mL of water
- This combination will produce a 0.293 molar IPA solution

To prepare an SDS stock solution

- Measure 0.005g of SDS onto a scale
- Add the measured SDS to 10mL of water

- This combination will produce a 0.00173 molar SDS solution

To prepare the capture reagent buffer for the antibodies

- Measure out 900 uL of ammonium acetate stock solution
- Add 20uL of stock IPA solution
- Add 80uL of stock SDS solution
- Add 1mg unlabeled lyophilized IgE antibody to solution
- Solution will be 1mg/mL of detector reagent to buffer [16].

After creating this solution deposit a small amount (enough to saturate the detection region) of solution onto detection region. Heat the chip to 35 – 40 degrees C for 2 hours to affix the capture reagents to the detection region [3].

After affixing the capture reagents to the detection region, it is important to block the rest of the membrane. The purpose of blocking the membrane is to prevent the target analytes, as well as the detection reagents from binding to random sites and indicating a false positive result [3]. To block the membrane of the chip, the following steps were used.

- Measure out 0.6g of BSA on a scale
- Deposit measured BSA into 30mL of reagent quality water
- Solution will be 2%w/v of BSA [3]

Immerse the chip into this blocking solution for 1 – 15 minutes.

Next, a washing step will be performed to remove any unattached BSA that could potentially cause flow problems that would null the chip [3]. To create a wash solution for the blocked membrane

- Add 300uL of sodium dibasic solution to 30mL of water
 - This will produce a wash solution that is 5mM
- Add 0.003g of SDS to the wash buffer
 - This will produce an SDS supplement of 0.01%w/v
- Solution will be 5mM supplemented with 0.01%w/v SDS [3]

Immerse the chip in this solution for 5 – 15 minutes. Make sure that the chip contacts this washing solution for a longer period of time than the blocking solution. To dry the chip after the blocking and washing, heat the chip to 35 – 40 degrees C for 2 hours. This drying step will help remove any complex water that has formed on the surface of the membrane [3].

The buffer for the conjugate pad should be simple. Millipore suggests that if using gold colloidal particles to buffer the conjugate solution using 2mM borate buffer (pH 7) that has been supplemented with 1 – 10% sucrose [3]. Since there was no indication as to which buffers to use for FITC, it was decided that BSA would suffice. To prepare the conjugate pad buffer

- Place 0.5mg FITC labeled IgE antibody (the detector reagent) sample in 1mL of water and allow it to dissolve
- Measure out and dissolve 2.657 grams of BSA into 19.92 mL water.
- Measure out and dissolve 0.001 grams of sucrose into the BSA solution

- Transfer 80uL of FITC labeled antibody sample solution into the water.
- The conjugate solution will have a concentration of 2ug/mL [16] within a 2mM buffer solution supplemented with 10% sucrose [3]

Dip the conjugate pad into solution and allow it to become completely submerged. Lay the pad on a non-absorbent surface and dry at 37 degrees Celsius for two hours [3]. Two antibody chips were performed using this “low” conjugate concentration. To prepare a “medium concentration” solution, add 920uL more FITC labeled antibody solution to buffer solution. This will produce a solution that is 22ug/mL (Conjugate /Solution). Four antibody chips were performed using the “medium” conjugate concentration. To prepare a “high concentration” solution, pipette out 1mL of “medium concentration conjugate solution. Add 0.5mg FITC labeled antibody into the solution and allow it to dissolve. This will produce a solution that is 522ug/mL (Conjugate/Solution). Four antibody chips were performed using the “high” concentration buffer solution. The medium and high concentration pads are to be dried in the same manner as the low concentration pads.

For the purposes of proving the concept of creating the paper microfluidic chip using a nitrocellulose platform, an idealized patient sample consisting only of the target analyte and water was created. To create the sample solution

- Dissolve 50ug of IgE into 50mL reagent quality water

This will produce 1ug/mL concentration of IgE [16].

Performing the Antibody Experiments

Place the conjugate pad onto the large rectangle at the bottom of the chip design. Deposit sample solution until the pad becomes completely absorbed with solution. Wait for the solution to flow to the detection region of the chip, placing more solution on the pad if necessary. Once the fluid has completely routed through the channels of the chip, image the chip using a FITC filter on a fluorescence microscope.

Aptamer Experiments: 2D Paper Microfluidic Devices

The aptamer sequence used for this experiment was 5 –GCGCGGGGCACGTTTATCCGTCCCTCCTAGTGGCGTGCCCCGCGC – 3 [16]. All steps to create stock solutions, conjugate pads, etc. can be found in the antibody section of this report. The only deviation lies in the preparation of the detection region buffer. That protocol is as follows.

- Add 23,390 uL of water to the aptamer container provided from the manufacturer.
- Take 500uL of this sample and add it to a new container that will be used for the duration of the solution creation.
 - The rest of the sample was preserved in the freezer as 1mL aliquots.
- Add 460uL of ammonium acetate stock solution
- Add 20uL of stock IPA solution
- Add 20uL of stock SDS solution

This will produce a buffer solution with a concentration of 7.09mM [3]. The solution overall will have a concentration of 1mg/mL of detector reagent to buffer [16]. Deposit a small amount (enough to saturate the detection region) of solution onto detection region. Heat the chip to 80 degrees C for 2 hours [20]. All blocking, washing, drying, and

conjugate pad preparation steps were performed in the same manner as was done for the antibody experiments.

Performing the Aptamer Experiments

The aptamer experiments will be performed in the exact same fashion at the antibody experiments. Two chips will be performed with “low” conjugate concentrations, four with “medium”, and four with “high”.

RESULTS

Paper Characterization Experiments: 2D Paper Microfluidic Devices

Characterization of Development Time

The following graph shows the data that was collected from the laboratory for the development time characterization experiments.

Figure 44: Raw Data from Development Time Characterization Experiments

For a complete detailed chart of specific development times that were recorded during the course of the experiment, please turn your attention to the Appendix A: Raw Data Section of this thesis report.

Once the statistical transformation had been completed (please see Appendix B: Detailed Statistical Analysis for details), Minitab was used to generate the following output after performing a linear regression:

Regression Equation

$$\ln \text{ development} = 4.37428 + 0.0892568 \frac{1}{\text{width}} + 7.34762 \text{ Length (inches)} - 0.44306 \frac{1}{\text{width}} \text{Length (inches)}$$

Coefficients

Term	Coef	SE Coef	T	P
Constant	4.37428	0.56502	7.74187	0.000
1/width	0.08926	0.03955	2.25694	0.033
Length (inches)	7.34762	2.05643	3.57300	0.001
1/width*Length (inches)	-0.44306	0.14803	-2.99307	0.006

Summary of Model

S = 0.463210 R-Sq = 37.83% R-Sq(adj) = 30.37%
PRESS = 6.98658 R-Sq(pred) = 19.03%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	3.26418	3.26418	1.08806	5.0710	0.0070243
1/width	1	0.36020	1.09294	1.09294	5.0938	0.0330008
Length (inches)	1	0.98183	2.73918	2.73918	12.7663	0.0014695
1/width*Length (inches)	1	1.92216	1.92216	1.92216	8.9585	0.0061395
Error	25	5.36408	5.36408	0.21456		
Lack-of-Fit	12	4.82649	4.82649	0.40221	9.7262	0.0001243
Pure Error	13	0.53759	0.53759	0.04135		
Total	28	8.62827				

Fits and Diagnostics for Unusual Observations

Obs	ln development	Fit	SE Fit	Residual	St Resid	
26	4.90520	5.85088	0.120114	-0.945677	-2.11388	R

R denotes an observation with a large standardized residual.

The conclusions and inferences that can be made from this statistical analysis will be discussed in more detail in the discussion section of this thesis report.

Paper Characterization

The following figures show the chips that were created for the purposes of paper characterization after the colorimetric tests had been performed. This first figure shows the bibulous paper chips.

Figure 45: Bibulous paper chips for paper characterization

The chip with a single channel was unable to allow for fluid wicking to the detection region. The chip with two channels was able to allow for fluid wicking to both detection regions. The chip with three channels was only able to allow for fluid wicking to one detection region. The following figure shows the chips that were created with unbacked nitrocellulose.

Figure 46: Unbacked nitrocellulose chips for paper characterization experiments

From the figure, we can see that the fluid was never able to penetrate into the pores of this specific paper type. The following figure shows the paper chips that were created using the Millipore backed nitrocellulose paper (HF13504XSS).

Figure 47: Millipore backed nitrocellulose for paper characterization experiments

We can see from the figure that the fluid was able to successfully route through all channels and show up in each detection region with the exception of the 0.8mm channel width chip with three channels. Based on the results of this experiment, the Millipore nitrocellulose was the paper used for all future chip manufacturing that will be discussed in this results section.

Colorimetric Experiments: 3D Paper Microfluidic Devices

Printing Method Development

As mentioned before, there were multiple printing methods that were considered for the manufacturing of paper microfluidic chips. The first method was to simply follow the “unbacked” protocol. This method was completely unsuccessful. The unbacked nitrocellulose is much more fragile than the backed nitrocellulose. Printing the paper in this manner resulted in the paper ripping within the printer and becoming jammed within the rollers. After one disastrous failed attempt, this method was immediately abandoned.

The second method involved using a paper template and using the electrostatic interactions between the nitrocellulose layers to keep the papers together within the printer. This method was initially a success. The layers were able to be printed in this fashion for about 4 – 5 iterations before the unbacked layers began slipping into the rollers of the printer and jamming the machine. After multiple iterations of this failure occurring, this method was also abandoned due to its lack of repeatability.

The third and final method included the paper template with tape for attachment purposes. This method worked remarkably well. The unbacked layers never became unattached from the template, and this method was used countless times with consistent and reliable results. Every time this method was used, the unbacked layers came out with the appropriate channels printed on them without any printer issues. The only drawback with this method is that sometimes the unbacked layers will come out looking slightly “creased”. This effect is completely eliminated after heating of the individual layers. Since this creasing can be successfully eliminated without any consequences, and since the method itself was so repeatable and reliable, this method was used for all future chip manufacturing.

Bridging Method Development

As mentioned before, there were three bridging methods that were considered for the purposes of manufacturing 3D paper microfluidic chips. The first method included manufacturing the paste using acetone as a dissolving agent and allowing for the bridging layer to set overnight before use. When this plan was used, the grinded paper would often form tight, dense, clumps. These clumps would have to be broken upon manufacturing using a stirring rod. Often, these clumps wouldn't be easily broken into

smaller entities, and they would often poke through the unbacked layers of nitrocellulose and create holes. Chips manufactured using this method also had extremely slow detection times, indicating that the bridging wasn't occurring effectively. This method was eventually abandoned for these reasons.

The second method involved cutting the paper to the size of the flow holes using scissors. Upon manufacturing, these small pieces of nitrocellulose would often stick to the operators fingers. The process of cutting the paper to size, while dealing with the electrostatic interaction was also frustrating and time consuming. This method was extremely cumbersome, and chips created using this method had extremely long detection times (most were abandoned due to lack of detection after long periods). This method of detection was also eventually abandoned.

The third method involved making "fresh" paste by grinding the paper in water and using the paste immediately after use. This method eliminated many problems that were seen with the first two methods. Since the paste was fresh, it was extremely pliable and didn't create holes in the unbacked nitrocellulose. Since it contained a small amount of water, it didn't stick to the operator during assembly, which made the entire process more efficient. This method of bridging also produced the chips with the most accurate and time efficient development times. For these reasons, this method was used for all future chip manufacturing.

3D Chip Development Time and Fluid Routing

Successful results were found in chips that were manufactured using the most efficient printing and bridging method. All other combinations of methods produced chips that took hours to detect, if they did at all. The chip that was designed for this

experiment (show in earlier figures) has a longer channel lengths on the top and bottom assays as compared to the middle assays. Given this fact, one of the criteria for success was that this specific chip produces simultaneous detection of the middle assays, followed by the top and bottom assays. The only chip to produce this effect successfully was manufactured using the optimum techniques. The figure below shows the middle two assays clearly detecting before the top and bottom.

Figure 48: Middle assays detected successfully. Top and bottom assays beginning to show in the detection layer

The following figure shows the top and bottom assays getting closer to detection.

Figure 49: Top and bottom assays getting closer to successful detection

Finally, the next figure shows all four assays detecting successfully.

Figure 50: Successful detection of all four assays

It is important to note that this was the only chip that was able to produce completely successful results. However, even with accurate detection in the specified order, the development time took over an hour to complete.

Antibody Experiments: 2D Paper Microfluidic Devices

The antibody chips were imaged using a fluorescence microscope configured to show FITC labeled substances and the program Q Capture Pro. The library of images for each chip can be found in Appendix E: Antibody/Aptamer Chip Images. The following table shows the general results that were obtained for the antibody experiments.

Table I: Antibody Experiment Results

Chip Number	Chip Type	[Detector Reagent]	Reached Detection	Detected Successfully	Notes
1	Antibody	Low	Yes	Yes	
2	Antibody	Low	Yes	Yes	No Running
3	Antibody	Medium	No	No	No Running
4	Antibody	Medium	Yes	Yes	
5	Antibody	Medium	Yes	Yes	
6	Antibody	Medium	Yes	Yes	
7	Antibody	High	Yes	Yes	
8	Antibody	High	Yes	Yes	
9	Antibody	High	Yes	Yes	No Running
10	Antibody	High (DROP)	N/A	Yes	No Running

It is important to note that chip 10 was a control chip that didn't contain a conjugate pad. The reagents themselves were spotted directly into the detection region. An image of this positive control sample specimen is shown in the figure below.

Figure 51: Antibody detection region, positive control (Chip 10)

Aptamer Experiments: 2D Paper Microfluidic Devices

The aptamer chips were imaged using a fluorescence microscope configured to show FITC labeled substances and the program Q Capture Pro. The library of images for each chip can be found in Appendix E: Antibody/Aptamer Chip Images. The following table shows the general results that were obtained for the aptamer experiments.

Table II: Aptamer Experiment Results

Chip Number	Chip Type	[Conjugate]	Reached Detection	Detected Successfully	Notes
11	Aptamer	Low	No	No	
12	Aptamer	Low	No	No	
13	Aptamer	Medium	No	No	
14	Aptamer	Medium	Yes	Yes	
15	Aptamer	Medium	No	No	No Running
16	Aptamer	Medium	Yes	Yes	
17	Aptamer	High	No	No	
18	Aptamer	High	No	No	No Running
19	Aptamer	High	Yes	Yes	No Running
20	Aptamer	High (DROP)	Yes	Yes	No Running

It is important to note that chip 20 was a control chip that didn't contain a conjugate pad. The reagents themselves were spotted directly into the detection region. An image of this positive control sample specimen is shown in the figure below.

Figure 52: Aptamer detection region, positive control (Chip 20)

DISCUSSION

Paper Characterization Experiments: 2D Paper Microfluidic Devices

Characterization of Development Time

From the output that was generated from Minitab we can see that the final model that was generated has validity when it comes to describing the flow characteristics of fluids within paper microfluidic chips. We can conclude that Length is a significant predictor of the transformed response variable $\ln(\text{Development time})$ ($F_{1,25} = 12.7663$ with a p – value of 0.0014695). The transformed predictor variable $1/\text{Width}$ is a significant predictor of the transformed response variable $\ln(\text{Development Time})$ ($F_{1,25} = 5.0938$ with a p – value of 0.0330008). The $1/\text{Width}$ and Length interaction is a significant predictor of the transformed response variable $\ln(\text{Development Time})$ ($F_{1,25} = 8.9585$ with a p – value of 0.0061395).

In conclusion, from the output generated in Minitab (specifically the coefficients table), we can see that, on average, the thinner channels with smaller lengths tend to produce development times that will be the most satisfactory when designing Paper Microfluidic Chips. For this analysis, lengths of 0.1 inches and widths of 0.15 inches will produce the chips with the shortest development time. The information gathered from this experiment was used later in the design process to produce paper microfluidic chips that require an adequate amount of development time to produce accurate results while still being feasible to use in the field of developing nations.

Paper Characterization

From the results presented, it is clear to see that the bibulous paper wasn't entirely consistent or reliable during the paper characterization experiments. It is clear to see from the image that the bibulous paper allows for the wax to travel large distances upon heating for penetration. This presents a problem that the channels decrease significantly in size upon heating in the oven. The sample introduced to the chip with a single channel never made it to the detection region and became trapped in the width of the channel. The sample introduced to the chip with three channels only made it to a single detection region. The only chip that was completely successful at routing the fluid was the chip with two channels. These results are unsatisfactory for the purposes of this experiment. We can conclude from the data that the bibulous paper isn't a platform that should be considered for other chips. This recommendation is made based on the fact that the wax spread a very large distance horizontally along the paper (as compared to other paper types) upon heating in the oven and that samples were never accurately or repeatedly detected.

The unbacked nitrocellulose was a complete failure. Although this paper allowed the wax to penetrate the thickness of the chip without much horizontal diffusion, the fluid itself was never able to penetrate into the paper's pores. Due to these results, this paper was never considered for use in other chip manufacturing procedures.

The backed nitrocellulose showed the most consistent results of all paper types tested. Upon heating, the wax showed very little movement horizontally across the paper. This is beneficial, as the lack of horizontal movement will help to ensure more accurate geometries can be produced from CAD files and maintained throughout

manufacturing. From the results, we can see that the sample was able to route through each chip and resulted in detection for every channel except for the thinnest channels on the chip with three detection areas. At this point it is still unknown as to why this happened. At the time of manufacturing, all chips used for this test were created from the same sheet of backed nitrocellulose. The only conclusion that can be drawn from this incident is that it was possible that the paper had somehow become contaminated and thus wouldn't allow for fluid routing. This conclusion was supported also by the fact that such an incident never occurred again during this entire stage of experimentation.

This experiment with the backed nitrocellulose was also used to characterize channel widths. It is important during chip manufacturing that the channel widths not be too wide so as to drastically increase development time. It is also important that the channels not be too thin so as to impede the flow. If the channels are too thin, it may also allow for the fluid to flow too rapidly so that the sample doesn't come into contact with its corresponding biochemical counterparts. This would result in inaccurate results being displayed by the chip itself (indicating a negative result when it should have been positive). Given all of these parameters in mind while assessing the data, it was decided that 1.6mm would be a perfect balance between channels that were too wide vs. too small. This channel width was used in other chip designs that occurred later in experimentation.

Overall, what we can take away from the paper characterization experiments is that using the backed nitrocellulose with 1.6 mm channels seems to produce the most desirable outcomes for our purposes.

Colorimetric Experiments: 3D Paper Microfluidic Devices

Printing Method Development

The printing method development yielded an obvious choice for future chip development. The taping/template method should be used for all subsequent chip development. It is the only method that was attempted that produces consistent results without risk of causing damage to the materials or to the printer. It may be desirable to develop a more efficient way to print the unbacked layers. While the taping method is effective and produces layers of desirable quality, the process itself is quite time consuming. It takes a significant period of time to attach the small squares to each corner of the paper. Reducing the time to create the unbacked layers would significantly make the entire process more effective and would be a necessary development if a larger quantity of chips were to be produced.

Bridging Method Development

The bridging layer experiments yielded an obvious top choice to be used for subsequent chip manufacturing. The “fresh paste” method was a quick and accurate way to make a paste that would allow for bridging from one layer of paper to another. This method was the only method that was able to produce a 3D chip that routed fluid at the rates and in the order that it was designed and intended to produce. However, it should be noted that accurate flow through a 3D device was only performed successfully on one occasion. It is clear that more research needs to go into the bridging layer technique so that it may be optimized and used for future projects in a more efficient manner. Only once the bridging layer is optimized, can all other 3D projects be a complete success. However, given the goals of this specific thesis project, the bridging layer that was

created performed to its intended purpose and successfully showed that the technique, while not always the most efficient and reliable, produces successful results for our purposes.

The method for bridging that was considered the most successful does come with some limitations. First, the act of breaking down the paper using the mortar and pestle is time consuming. Since the paper itself is dissolved in water, there aren't any degradation processes happening within the solution to help speed up the process. Instead, this method relies on purely mechanical breakdown of the paper. Relying on this mechanism alone makes the process more time consuming than it would be otherwise. It may be wise to consider dissolving the paper in another solution, such as acetone. Were processes using such a solution to be considered, great care would need to be taken to ensure that the addition of the chemical wouldn't have a detrimental impact on the performance of the reagents that would be used for the assay. Another method that could be considered is to revisit the paper cutting technique. A way to improve upon this process would be to use a hole-punch to create the circles in a much more timely and less cumbersome manner. It is possible that stacking multiple pieces of the punches paper to create a thicker layer would also make the technique more effective.

Overall, while a bridging layer was successfully created for the purposes of this thesis project, a more efficient method should be created to help make the manufacturing process more streamlined and effective. This new method would need to accurately allow fluid to bridge through a 3D assay in an amount of time that is feasible for diagnostics in remote locations.

3D Chip Development Time and Fluid Routing

During this experiment, a 3D microfluidic device was successfully created to demonstrate that fluid routing behaviors could be engineered to produce accurate detection in a specific order. While this experiment successfully showed that the process is indeed possible, it is important to note that in the end, only one chip was developed to be considered completely successful. In order for the 3D platforms to be considered a complete success overall, recommendations for changes in bridging techniques and printing techniques (as discussed previously) will need to be considered. Changes to these two specific areas of development will help to shorten development time and ensure that all assays perform successfully during each of the iterations of the experiment. Once these changes have been made, and consistently accurate and satisfactory results have been obtained, then the platforms can be expanded to contain biochemical agents for actual biomarker detection.

Antibody Experiments: 2D Paper Microfluidic Devices

Since antibodies are traditionally used as biochemical agents in lateral flow assays, it was expected that these chips would perform optimally and be used as a comparison for the aptamer experiments. As we can see from the results in Table I, all chips performed optimally with the exception of chip 3. Chip 3 never reached the detection region, and the sample became trapped within the channel. The figure below shows the end of the channel on chip 3 where the sample stopped moving.

Figure 53: Chip 3 - Sample entrapment at channel end

In the figure it is clear to see that there is a sharp harsh line that cuts through the entire width of the channel. This was the first time that this phenomenon had occurred during this experimentation. When the chip was examined, it was noted that the paper itself has actually split and become unattached from the backing. Since the paper split from the backing formed a void across the channel length, it makes sense that the sample itself was never able to cross this threshold and reach the detection region. This splitting behavior was never noticed when the paper was tested with colorimetric reagents. When the other chips were inspected, it was noted that sections containing this splitting behavior were present on other antibody chips as well. The difference between the other chips and chip 3, was that chip 3 was the only chip that failed with this defect present. Even though only one chip failed, the fact that these defects are present on multiple chips is a concern. Should these chips be created and tested again in further experimentation, it would be wise to consider a more robust membrane material that will be able to withstand the addition of the chemical reagents.

All other chips that were tested for this portion of the experimentation produced satisfactory results. Successful detection was noted by an increase (visible at the lowest magnification of the microscope) in fluorescence present within the detection region. An example of such successful detection is shown below.

Figure 54: Chip 2 - Successful detection example

In this image, the channel of the chip would be located to the right of the detection region (not present within this image) with the overflow region present on the left (not present within this image). It is clear to see that the sample itself has flown through the detection region and congregated in the area of the detection region where the capture reagents are present leaving other areas free of signal. The fact that there are areas of the chip that are completely free of sample is a good indication that the blocking methods that were performed on each of the chips were successful. If blocking were not successful, the sample would have appeared to have a bright green color on all areas of the chip as the unspecific binding would have produced a constant signal everywhere that it was capable of attaching.

An aspect of running antibody chips that proved to be challenging was the application of the capture reagents to the membrane itself. For the purposes of this experiment, only micropipettes were available for depositing the reagents. Under factory conditions, a striper, or some other precise application instrument would be used for the placement of these reagents. Since obtaining this equipment was fiscally impossible, the smallest micropipette was used and the sample was deposited as carefully as possible onto the membrane. This method of depositing the reagents is far from ideal. This method causes too much of the reagent solution to be deposited and causes some runoff into the channels and overflow region. Only on a few occasions was runoff completely avoided (noted in the results tables). Theoretically, even with runoff the tests can still run successfully. Since at the time of reagent application the membrane isn't blocked, when the sample solution spreads, the concentration of detection reagents will decrease drastically the farther the sample travels from the initial point of application. Thus, we should still get a clear bright signal in the area of deposition, with a slightly "quieter" signal surrounding it. An example of this occurring on a chip with runoff is shown below in the following figure.

Figure 55: Chip 1 - Detection with runoff present

This figure shows the end of the detection region with a portion of the sample runoff region on the left of the image. When depositing reagents onto the detection region of this specific chip, there was some runoff into the overflow region. It is clear to see on the right side of the figure that there is a higher concentration of FITC present with a sharp line indicating a decrease in signal as the sample travels closer to the overflow region. This figure shows nicely that there is a detectable difference in the concentrations of capture reagents present when runoff has occurred. It also shows that even in the non-ideal conditions that this experiment was performed in; the proof of concept is still shown accurately.

Overall, the antibody experiments were extremely valuable. They showed successful blocking of the membrane, sample detection using traditional antibodies in an ideal case, and sample detection using traditional antibodies in a non-ideal case. The antibody experiments as a whole can be considered a success.

Aptamer Experiments: 2D Paper Microfluidic Devices

It was definitely more of a challenge to receive positive results with the aptamers than it was with the antibodies. The splitting of the nitrocellulose from the backing that occurred with the antibodies occurred much more frequently with aptamers. In some cases, large tears would completely separate portions of the paper from the backing after the tests had been performed. These tears were much more apparent and obvious with the naked eye than when compared to the antibody chips. The presence of these large tears definitely seemed to severely increase the failure rate for the chips. There were only 4 chips that were considered a success out of the total 10.

There was another strange phenomenon that occurred during the aptamer experiments that didn't occur with the antibodies. The aptamer reagents would sometimes flow up the channels and displace deposited wax to produce wider channel widths. An example of this is shown in the figure below.

Figure 56: Chip 15 - Increased channel width with the application of aptamer reagents

The presence of this wax degradation and channel widening is definitely an indication that there are some severe biochemical reactions occurring between the aptamer solution and the nitrocellulose chip. Since the same reagents are present in the buffers of both the antibody and aptamer capture reagents, we can conclude that the entity most likely to blame for the cause of this phenomenon is the aptamers themselves. Whether it was the reaction that the aptamers had with the deposited wax, or this phenomenon combined with the production of cracks within the channels, it is clear that one of these two options was the main cause for such high failure rates seen for the aptamer chips.

Even though there were such high failure rates seen with the aptamer chips, there were also some definite monumental successes. While we were unable to produce detection on chips with absolutely no runoff of the initial deposited reagents, we were able to produce images that clearly show detection in the less than ideal case (where reagent runoff upon application of capture reagents is present). An example of such a success is shown below.

Figure 57: Chip 14 - Detection region concentration differentiation

This figure shows an area slightly to the left of the detection region. The channel leading to the detection region of this chip is to the right of the image (not shown in the image) and the overflow region is to the left of the image (not shown in the image). When depositing reagents onto the detection region of this specific chip, there was some runoff into the overflow region. It is clear to see on the right side of the figure that there is a higher concentration of FITC present with a sharp line indicating a decrease in signal as the sample travels closer to the overflow region. This figure shows nicely that there is a detectable difference in the concentrations of capture reagents present when runoff has occurred. It also shows that even in the non-ideal conditions that this experiment was performed in; the proof of concept is still shown accurately.

Overall, the goals of the aptamer experiments were successfully carried out. Despite setbacks in development, we were able to show accurate detection on multiple chips, therefore demonstrating that aptamers can be used as capture reagents on paper microfluidic platforms based in nitrocellulose.

Conclusions and Future Work

Overall, the experiments for this thesis project have been successfully carried out. For the 3D colorimetric experiments, we have successfully shown that fluid routing and timing can be engineered and controlled to produce specific outcomes. For future projects, the printing methods for unbacked paper could be streamlined to be more efficient, and more effective bridging layers need to be explored.

The antibody experiments have successfully served as a baseline to show that lateral flow assays can be created and performed using proper biochemistry on a

nitrocellulose platform. Future work could be done to scale the assay up to a 3D model so that multiple samples can be tested at the same time.

The aptamer experiments have successfully shown that aptamers can be used as capture reagents on paper microfluidic chips. Future iteration projects should consider scaling to a 3D model so that multiple samples can be tested at the same time. Also, it would be beneficial to consider different membranes for the analysis. This project showed that while this specific platform has the ability to produce successful results, that other membranes should be considered to ensure that a higher percentage of tests will be successful in the future.

Demonstrating more robust 3D assays is a necessary next step. A method to help streamline the manufacturing process for these assays would be the addition of an alignment technique. Using pins to align the layers during placement would allow for more accurate construction, and in turn more accurate detection capabilities. An example of such an alignment technique was developed and is shown in the following figure.

Figure 58: Alignment technique prototype

This technique would require syringe tips to be placed in the holes at the four corners of the device. These tips would line up with small holes that would be created in the corner of each paper and tape layer. Manufacturing the chips would include slipping these syringe tips through the holes to ensure that each layer was aligned correctly.

If the work done in this thesis experiment was continued and used to develop a robust system for IgE detection, then some drastic next steps could be taken. Given the successful development of the platform, these chips could be sent to foreign nations for the purposes of diagnosing patients in remote locations. This remote diagnosis would require a sort of “reader” to analyze the results in the field. For the purposes of providing an example for visualization, a rendering of this device is shown in the figure below.

Figure 59: Prototype paper microfluidic chip reader

This reader could input the sample chip via a small slit on the right hand side of the device. The optical and electrical read outputs could be seen on the screen. A USB port could be added at the bottom of the device to allow it to be connected to a standard desktop computer. If this platform were developed to the point of creating such a device, the diagnostic field as we know it would be revolutionized.

Overall, we have successfully shown progress in the field of paper microfluidics. We have shown that we can use detector reagents in the form of aptamers to diagnose IgE producing respiratory issues in ideal cases without excessive sample preparation. General next steps would include allotting for sample pretreatment within the chip's sample pad, and ultimately testing the chips on subjects with asthma to see if an accurate diagnosis can be produced. Overall, this thesis project has helped develop the field of paper microfluidics and will hopefully help to revolutionize the industry and allow for more accurate diagnosis across developing nations.

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APPENDIX A: RAW DATA

Characterization of Development Time

The following table shows the raw data that was collected for the development time experiment.

Table III: Characterization of Development Time Raw Data

Paper	Length (in)	Width (in)	Development Time (s)
1	0.1	0.15	197.82
1	0.1	0.1	237.83
1	0.1	0.05	312.63
1	0.1	0.06	286.84
1	0.2	0.15	65.35
1	0.2	0.1	240.18
1	0.2	0.05	510.97
1	0.2	0.06	494.45
1	0.3	0.15	417.14
1	0.3	0.1	664.3
1	0.3	0.05	400.77
1	0.3	0.06	48.45
1	0.4	0.15	852.89
1	0.4	0.1	869.37
1	0.4	0.05	319.14
1	0.4	0.06	169.03
2	0.1	0.15	321.56
2	0.1	0.1	498.75
2	0.1	0.05	274.3
2	0.1	0.06	218.19
2	0.2	0.15	246.24
2	0.2	0.1	232.34
2	0.2	0.05	617.77
2	0.2	0.06	522.39
2	0.3	0.15	448.14
2	0.3	0.1	760.35
2	0.3	0.05	561.54
2	0.3	0.06	134.99
2	0.4	0.15	973
2	0.4	0.1	815.42
2	0.4	0.05	997.23
2	0.4	0.06	167.95

APPENDIX B: DETAILED STATISTICAL ANALYSIS

Characterization of Development Time

First, the model Development Time = Length + Width + Paper was generated using the general linear model command in Minitab. The following ANOVA table was generated in Minitab.

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	614343	614343	204781	3.59794	0.025723
Width	1	22587	22587	22587	0.39685	0.533830
Length	1	501124	501124	501124	8.80459	0.006090
Paper	1	90632	90632	90632	1.59237	0.217399
Error	28	1593654	1593654	56916		
Total	31	2207996				

As we can see, width doesn't seem to be a significant predictor ($F_{1,28} = 0.39685$ with a p – value of 0.533830). Before we completely rule out width we need to check out a possible interaction that could be significant between the predictor variables length and width. The model Development Time = Length + Width + Paper + Length*Width was generated using the general linear model command in Minitab. The following ANOVA table was generated.

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	615429	615429	153857	2.60846	0.057837
Width	1	22587	22587	22587	0.38293	0.541223
Length	1	501124	501124	501124	8.49593	0.007073
Paper	1	90632	22755	22755	0.38578	0.539735
Width*Paper	1	1086	1086	1086	0.01841	0.893070
Error	27	1592568	1592568	58984		
Total	31	2207996				

This model produces a non-significant regression statistic ($F_{4,27} = 2.60846$ with a p – value of 0.05837). Let's take a look at the residual plot for the predictor variable width.

Figure 60: Standardized Residuals plotted against Width generated using the model Development Time = Length + Width + Paper + Length*Width

It looks as though we have a situation where the residual plot is skewed to the right. In order to deal with this issue, let's try using a transformed predictor variable $1/\text{width}$. Next, we fit the model: Development Time = Length + $1/\text{Width}$ + Paper + Length* $1/\text{Width}$. This model generates the following ANOVA table.

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	4	865780	865780	216445	4.3540	0.007595
Paper	1	90632	90632	90632	1.8231	0.188150
$1/\text{width}$	1	22172	157874	157874	3.1758	0.085988
Length	1	501124	528441	528441	10.6301	0.003007
$1/\text{width} \times \text{Length}$	1	251852	251852	251852	5.0662	0.032743
Error	27	1342217	1342217	49712		
Total	31	2207996				

We can see from this output that the interaction between length and $1/\text{width}$ is significant.

The following graph shows the residual plot for $1/\text{width}$.

Figure 61: Standardized Residuals plotted against 1/Width generated using the model $\text{Development Time} = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$

The plot looks much more homogeneous than it did before, although now we can see an extreme outlier in the top right hand corner of the plot. Let's remove the point from our analysis so that the data is less skewed. Let's also remove our blocking variable paper. We can do this because up to this point since the variable fails to be significant in any of our previous analysis and it may be doing more harm than good being in our model ($F_{1,27} = 1.8231$ with a p – value of 0.188150). Now we can fit the model $\text{Development Time} = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$. The following ANOVA table was generated from this model.

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	882048	882048	294016	7.9536	0.0005867
1/width	1	82068	273666	273666	7.4031	0.0112540
Length	1	326725	723873	723873	19.5818	0.0001426
1/width*Length	1	473254	473254	473254	12.8022	0.0013358
Error	27	998099	998099	36967		
Lack-of-Fit	12	900397	900397	75033	11.5197	0.0000174
Pure Error	15	97702	97702	6513		
Total	30	1880147				

We can see that all of our predictor variables are now significant! But we have a problem with our normality of the residuals (plot shown below).

Figure 62: Normality Test Plot of Standardized Residuals generated using the model $\text{Development Time} = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$

In order to help increase the normality of our residuals, let's try transforming the response variable by fitting the following model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$. The normality plot of this new model is shown below.

Figure 63: Normality Test Plot of the Standardized Residuals generated using the model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$

We can now see that we have some outliers present at the bottom left hand corner of our plot. Let's remove these points and fit the model one last time.

The final model that was generated uses the following word equation:

$$\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$$

First, let's look at the diagnostic graphs generated for this model. The following graph shows the normality plot of the residuals as generated in Minitab.

Figure 64: Normality Test Plot of the Standardized Residuals generated using the model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$ with outliers removed.

As we can see from the output, we have a p – value of 0.118 meaning that we fail to generate a significant amount of evidence to show that the standardized residuals do not follow a normal distribution. The following graph shows the standardized residuals vs. the fits generated by the model using the capabilities of Minitab.

Figure 65: Standardized Residuals plotted against the Fitted Values generated using the model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$ with outliers removed.

We can see that this is the most uniformly distributed residual plot that was generated for fitted values for any model that was tested using Minitab. The following graph shows a histogram of the residuals that were generated for this model using Minitab.

Figure 66: Histogram of the Standardized Residuals generated using the model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$ with outliers removed.

We can see from the histogram that the standardized residuals seem to follow a relatively normal distribution, which is a requirement for performing a general linear model. Finally, the following graphs show the residuals when plotted against the predictor variables. The first graph shows the residuals plotted against 1/width.

Figure 67: Standardized Residuals plotted against 1/Width generated using the model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$ with outliers removed.

The second graph shows the residuals plotted against length.

Figure 68: Standardized Residuals plotted against Length generated using the model $\ln(\text{Development Time}) = \text{Length} + 1/\text{Width} + \text{Length} * 1/\text{Width}$ with outliers removed.

When looking at the graphs of the residuals plotted against the predictor variables we can see that we have relatively homogeneous variances, specifically, the most homogenous when considering all of the different analyses that have been tested.

With the diagnostic graphs showing results that allow us to make the necessary assumptions for a general linear regression, we can now look at the generated statistics and come to some solid conclusions. When performing a linear regression using this equation Minitab generates the following output.

General Regression Analysis: In development versus 1/width, Length (inches)

Regression Equation

In development = 4.37428 + 0.0892568 1/width + 7.34762 Length (inches) - 0.44306 1/width*Length (inches)

Coefficients

Term	Coef	SE Coef	T	P
Constant	4.37428	0.56502	7.74187	0.000
1/width	0.08926	0.03955	2.25694	0.033
Length (inches)	7.34762	2.05643	3.57300	0.001
1/width*Length (inches)	-0.44306	0.14803	-2.99307	0.006

Summary of Model

S = 0.463210 R-Sq = 37.83% R-Sq(adj) = 30.37%
PRESS = 6.98658 R-Sq(pred) = 19.03%

Analysis of Variance

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Regression	3	3.26418	3.26418	1.08806	5.0710	0.0070243
1/width	1	0.36020	1.09294	1.09294	5.0938	0.0330008
Length (inches)	1	0.98183	2.73918	2.73918	12.7663	0.0014695
1/width*Length (inches)	1	1.92216	1.92216	1.92216	8.9585	0.0061395
Error	25	5.36408	5.36408	0.21456		
Lack-of-Fit	12	4.82649	4.82649	0.40221	9.7262	0.0001243
Pure Error	13	0.53759	0.53759	0.04135		
Total	28	8.62827				

Fits and Diagnostics for Unusual Observations

Obs	In development	Fit	SE Fit	Residual	St Resid	R
26	4.90520	5.85088	0.120114	-0.945677	-2.11388	R

R denotes an observation with a large standardized residual.

We can see that this final model contains all significant predictors with appropriate assumptions validated in previous analysis.

APPENDIX C: DRAWING FILES AND DIMENSIONS

Smaller Colorimetric Chip

The following drawing shows the dimensions of the backed layer of the smaller colorimetric chip. The dimensions are reported in millimeters.

Figure 69: Dimensions of smaller backed paper layer in millimeters

The following drawing shows the dimensions of the first layer of tape of the smaller colorimetric chip. The dimensions are reported in millimeters.

Figure 70: Dimensions of smaller first tape layer in millimeters

The following drawing shows the dimensions of the first layer of unbacked paper of the smaller colorimetric chip. The dimensions are reported in millimeters.

Figure 71: Dimensions of smaller first unbacked paper layer in millimeters

The following drawing shows the dimensions of the second layer of tape of the smaller colorimetric chip. The dimensions are reported in millimeters.

Figure 72: Dimensions of smaller second tape layer in millimeters

The following drawing shows the dimensions of the second layer of unbacked paper of the smaller colorimetric chip. The dimensions are reported in millimeters.

Figure 73: Dimensions of smaller second unbacked paper layer in millimeters

The following drawing shows the dimensions of the third layer of double sided tape of the smaller colorimetric chip. The dimensions are reported in millimeters.

Figure 74: Dimensions of smaller third tape layer in millimeters

Larger Colorimetric Chip

The following drawing shows the dimensions of the backed layer of the larger colorimetric chip. The dimensions are reported in millimeters.

Figure 75: Dimensions of larger backed paper layer in millimeters

The following drawing shows the dimensions of the first layer of tape of the larger colorimetric chip. The dimensions are reported in millimeters.

Figure 76: Dimensions of larger first tape layer in millimeters

The following drawing shows the dimensions of the first layer of unbacked paper of the larger colorimetric chip. The dimensions are reported in millimeters.

Figure 77: Dimensions of larger first unbacked paper layer in millimeters

The following drawing shows the dimensions of the second layer of tape of the larger colorimetric chip. The dimensions are reported in millimeters.

Figure 78: Dimensions of larger second tape layer in millimeters

The following drawing shows the dimensions of the second layer of unbacked paper of the larger colorimetric chip. The dimensions are reported in millimeters.

Figure 79: Dimensions of larger second unbacked paper layer in millimeters

The following drawing shows the dimensions of the third layer of double sided tape of the larger colorimetric chip. The dimensions are reported in millimeters.

Figure 80: Dimensions of larger third tape layer in millimeters

Antibody/Aptamer Chip

The following drawing shows the dimensions of the chip that was used for the antibody and aptamer experiments. The dimensions are reported in millimeters.

Figure 81: Dimensions of the antibody/aptamer chip in millimeters

APPENDIX D: BIOCHEMISTRY CALCULATIONS

Preparation of the Stock Solutions

Preparation of the Alcohol stock solution calculations

$$\frac{0.100\text{mL}(\text{IPA})}{5\text{mL}(\text{water})} = 2\% \text{ solution}$$

$$0.100\text{mL}(\text{IPA}) * (91\%) = 0.091\text{mL}(\text{IPA})$$

$$0.091\text{mL}(\text{IPA}) * \left(\frac{0.986\text{g}(\text{IPA})}{1\text{mL}(\text{IPA})} \right) = 0.089726\text{g}(\text{IPA})$$

$$\left(\frac{0.089726\text{g}(\text{IPA})}{5.1\text{mL}(\text{solution})} \right) * \left(\frac{1000\text{mL}}{\text{L}} \right) * \left(\frac{1\text{mol}}{60.1\text{g}} \right) = \boxed{0.293\text{mol/L (IPA Stock)}}$$

Preparation of the Ammonium Acetate stock solution calculations

$$0.002\text{g}(\text{Amm.Ace.}) * \left(\frac{1\text{mol}}{77.08\text{g}} \right) * \left(\frac{1}{0.010\text{L}(\text{water})} \right) = \boxed{2.60\text{E-3 mol/L (Amm. Stock)}}$$

Preparation of the SDS stock solution calculations

$$0.005\text{g}(\text{SDS}) * \left(\frac{1\text{mol}(\text{SDS})}{288.38\text{g}(\text{SDS})} \right) * \left(\frac{1}{0.010\text{L}(\text{water})} \right) = \boxed{1.734\text{E-3 mol/L (SDS Stock)}}$$

Preparation of the Antibody Buffer

Addition of the IPA solution

$$\left(\frac{0.293\text{mol}}{\text{L}} \right) * (20\text{uL}) = \boxed{5.85\text{E-6 moles of IPA in the antibody buffer}}$$

Addition of the Ammonium Acetate solution

$$\left(\frac{2.60\text{E-3 mol}}{\text{L}} \right) * (900\text{uL}) = \boxed{2.34\text{E-6 moles of Amm. Ace. in Antibody Buffer}}$$

Addition of the SDS solution

$$\left(\frac{0.00173mol}{L}\right) * (80uL) = \boxed{1.387E-7 \text{ moles of SDS in Antibody Buffer}}$$

Total buffer concentration for antibody

$$\left(\frac{(5.85E-6 + 2.34E-6 + 1.387E-7)moles}{1mL(solution)}\right) = \left(\frac{8.31E-6moles}{1mL(solution)}\right) = \boxed{8.31mM}$$

Preparation of the Aptamer Buffer Solution

Dilution of the available aptamer

$$\left(\frac{13759g(Aptamer)}{mol}\right) * 3.4umol = 0.0467 \text{ grams}(Aptamer)$$

$$\left(\frac{1mg(Aptamer)}{0.5ml(Water)}\right) = \frac{0.04678g(Aptamer)}{0.02339mL(Water)}$$

Addition of the Ammonium Acetate

$$\left(\frac{0.00260mol}{L}\right) * (460E-6) = \boxed{1.196E-6 \text{ moles of Ammonium Acetate in Buffer}}$$

Addition of IPA Solution

$$\left(\frac{0.293mol}{L}\right) * (20E-6L) = \boxed{5.86E-6 \text{ moles of IPA in Buffer}}$$

Addition of SDS Solution

$$\left(\frac{0.00173mol}{L}\right) * (20E-6L) = \boxed{3.46E-8 \text{ moles of SDS in Buffer}}$$

Total Buffer Molarity

$$\left(\frac{(1.196E-6 + 5.86E-6 + 3.46E-8)moles}{0.001L(Solution)}\right) = \left(\frac{7.09mMol(Buffer)}{1L(Solution)}\right) = \boxed{7.09mM}$$

Conjugate Solution Preparation

Low Concentration Preparation

Dissolving the FITC labeled antibody

$$\left(\frac{500\mu g}{mL}\right)x = \left(\frac{2\mu g}{mL}\right)20mL \rightarrow x = 0.08mL \text{ of water to produce required concentration}$$

Addition of BSA to the conjugate solution

$$\left(\frac{2E-3moles}{L}\right) = \left(\frac{xmoles}{20mL}\right) \rightarrow x = 4E-5moles \text{ of BSA to produce required concentration}$$

$$4E-5moles * \left(\frac{66430gBSA}{moles}\right) = \boxed{2.2652 \text{ grams of BSA}}$$

Addition of sucrose to the conjugate solution

$$(4E-5moles) * 10\% = 4E-6moles(Sucrose)$$

$$4E-6moles * \left(\frac{342.3g}{mol}\right) = \boxed{0.0014g \text{ of Sucrose}}$$

Medium Concentration Preparation

$$0.920mL * \left(\frac{500\mu g}{mL}\right) = \left(\frac{x\mu g}{mL}\right) * 20.92mL \rightarrow \boxed{x = 22 \mu g/mL}$$

High Concentration Preparation

$$500\mu g + 22\mu g = \boxed{522\mu g/mL}$$

Blocking Solution Preparation

$$\left(\frac{x(gBSA)}{30mL(Water)}\right) = 2\% \rightarrow \boxed{x = 0.6g \text{ of BSA in } 30mL \text{ of water}}$$

Wash Solution Preparation

$$(0.5M) * (xmL) = 0.005M * (30mL) \rightarrow \boxed{x = 0.3mL \text{ of Sodium Dibasic Solution}}$$

$$\left(0.01\% \frac{v}{w}\right) = \left(\frac{xg}{30mL}\right)(100\%) \rightarrow \boxed{x = 0.003g \text{ of SDS Solution}}$$

Sample Solution Preparation

$$\left(\frac{1ug}{1mL}\right) = \left(\frac{50ug}{50mL}\right) \rightarrow \boxed{x = 50mL \text{ of Water}}$$

APPENDIX E: ANTIBODY/APTAMER CHIP IMAGES

Antibody Chip Images

Chip 1: Antibody with Low Concentration Conjugate Pad

The following image shows the entire view of Chip 1.

Figure 82: Chip 1

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 83: Chip 1 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 84: Chip 1 - Sample leaving the conjugate pad

The following image shows the channel leading to the sample detection region when viewed under a fluorescence microscope.

Figure 85: Chip 1 – Channel

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 86: Chip 1 - Entering the detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 87: Chip 1 - Leaving the detection region

Chip 2: Antibody with Low Concentration Conjugate Pad

The following image shows the entire view of Chip 2.

Figure 88: Chip 2

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 89: Chip 2 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 90: Chip 2 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 91: Chip 2 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 92: Chip 2 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 93: Chip 2 - Leaving the detection region

Chip 3: Antibody with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 3.

Figure 94: Chip 3

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 95: Chip 3 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 96: Chip 3 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 97: Chip 3 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 98: Chip 3 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 99: Chip 3 - Leaving the detection region

Chip 4: Antibody with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 4.

Figure 100: Chip 4

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 101: Chip 4 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 102: Chip 4 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 103: Chip 4 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 104: Chip 4 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 105: Chip 4 - Leaving the detection region

Chip 5: Antibody with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 5.

Figure 106: Chip 5

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 107: Chip 5 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 108: Chip 5 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 109: Chip 5 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 110: Chip 5 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 111: Chip 5 - Leaving the detection region

Chip 6: Antibody with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 6.

Figure 112: Chip 6

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 113: Chip 6 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 114: Chip 6 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 115: Chip 6 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 116: Chip 6 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 117: Chip 6 - Leaving the detection region

Chip 7: Antibody with High Concentration Conjugate Pad

The following image shows the entire view of Chip 7.

Figure 118: Chip 7

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 119: Chip 7 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 120: Chip 7 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 121: Chip 7 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 122: Chip 7 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 123: Chip 7 - Leaving the detection region

Chip 8: Antibody with High Concentration Conjugate Pad

The following image shows the entire view of Chip 8.

Figure 124: Chip 8

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 125: Chip 8 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 126: Chip 8 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 127: Chip 8 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 128: Chip 8 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 129: Chip 8 - Leaving the detection region

Chip 9: Antibody with High Concentration Conjugate Pad

The following image shows the entire view of Chip 9.

Figure 130: Chip 9

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 131: Chip 9 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 132: Chip 9 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 133: Chip 9 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 134: Chip 9 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 135: Chip 9 - Leaving the detection region

Chip 10: Antibody with High Concentration Drop

The following figure shows the entire view of Chip 10.

Figure 136: Chip 10

The following figure shows the detection region of the antibody control chip when viewed under a fluorescence microscope (Chip 10)

Figure 137: Chip 10 - Detection Region

Aptamer Chip Images

Chip 11: Aptamer with Low Concentration Conjugate Pad

The following image shows the entire view of Chip 11.

Figure 138: Chip 11

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 139: Chip 11 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 140: Chip 11 - Sample leaving the conjugate pad

The following image shows the channel leading to the detection region when viewed under a fluorescence microscope.

Figure 141: Chip 11 – Channel to detection region

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 142: Chip 11 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 143: Chip 11 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 144: Chip 11 - Leaving the detection region

Chip 12: Aptamer with Low Concentration Conjugate Pad

The following image shows the entire view of Chip 12.

Figure 145: Chip 12

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 146: Chip 12 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 147: Chip 12 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 148: Chip 12 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 149: Chip 12 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 150: Chip 12 - Leaving the detection region

The following image shows the overflow region when viewed under a fluorescence microscope.

Figure 151: Chip 12 - Overflow region

Chip 13: Aptamer with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 13.

Figure 152: Chip 13

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 153: Chip 13 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 154: Chip 13 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 155: Chip 13 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 156: Chip 13 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 157: Chip 13 - Leaving the detection region

The following image shows the overflow region when viewed under a fluorescence microscope.

Figure 158: Chip 13 - Overflow region

Chip 14: Aptamer with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 14.

Figure 159: Chip 14

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 160: Chip 14 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 161: Chip 14 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 162: Chip 14 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 163: Chip 14 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 164: Chip 14 - Leaving the detection region

Chip 15: Aptamer with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 15

Figure 165: Chip 15

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 166: Chip 15 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 167: Chip 15 - Sample leaving the conjugate pad

The following image shows the channel leading to the detection region when viewed under a fluorescence microscope.

Figure 168: Chip 15 – Channel to detection region

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 169: Chip 15 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 170: Chip 15 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 171: Chip 15 - Leaving the detection region

The following image shows the overflow region when viewed under a fluorescence microscope.

Figure 172: Chip 15 - Overflow region

Chip 16: Aptamer with Medium Concentration Conjugate Pad

The following image shows the entire view of Chip 16

Figure 173: Chip 16

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 174: Chip 16 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 175: Chip 16 - Sample leaving the conjugate pad

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 176: Chip 16 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 177: Chip 16 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 178: Chip 16 - Leaving the detection region

Chip 17: Aptamer with High Concentration Conjugate Pad

The following image shows the entire view of Chip 17.

Figure 179: Chip 17

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 180: Chip 17 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 181: Chip 17 - Sample leaving the conjugate pad

The following image shows the channel leading to the detection region when viewed under a fluorescence microscope.

Figure 182: Chip 17 – Channel to detection region

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 183: Chip 17 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 184: Chip 17 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 185: Chip 17 - Leaving the detection region

The following image shows the overflow region when viewed under a fluorescence microscope.

Figure 186: Chip 17 - Overflow region

Chip 18: Aptamer with High Concentration Conjugate Pad

The following image shows the entire view of Chip 18.

Figure 187: Chip 18

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 188: Chip 18 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 189: Chip 18 - Sample leaving the conjugate pad

The following image shows the channel leading to the detection region when viewed under a fluorescence microscope.

Figure 190: Chip 18 – Channel to detection region

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 191: Chip 18 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 192: Chip 18 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 193: Chip 18 - Leaving the detection region

The following image shows the overflow region when viewed under a fluorescence microscope.

Figure 194: Chip 18 - Overflow region

Chip 19: Aptamer with High Concentration Conjugate Pad

The following image shows the entire view of Chip 19.

Figure 195: Chip 19

The following image shows the conjugate pad when viewed using a fluorescence microscope.

Figure 196: Chip 19 - Conjugate pad

The following image shows the site where the sample leaves the conjugate pad when viewed under a fluorescence microscope.

Figure 197: Chip 19 - Sample leaving the conjugate pad

The following image shows the channel leading to the detection region when viewed under a fluorescence microscope.

Figure 198: Chip 19 – Channel to detection region

The following image shows the site where the channel enters the detection region when viewed under a fluorescence microscope.

Figure 199: Chip 19 - Entering the detection region

The following image shows a space in the middle of the detection region when viewed under a fluorescence microscope.

Figure 200: Chip 19 - Middle of detection region

The following image shows the site where the sample leaves the detection region to the overflow region when viewed under a fluorescence microscope.

Figure 201: Chip 19 - Leaving the detection region

The following image shows the overflow region when viewed under a fluorescence microscope.

Figure 202: Chip 19 - Overflow region

Chip 20: Antibody with High Concentration Drop

The following figure shows the entire view of Chip 20.

Figure 203: Chip20

The following figure shows the detection region of the aptamer control chip when viewed under a fluorescence microscope (Chip 20)

Figure 204: Chip 20 - Detection Region