Design and fabrication of a microsystem to rupture polymer vesicles

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Design and Fabrication of a Microsystem to Rupture Polymer Vesicles

January 2004
Design and Fabrication of a Microsystem to Rupture Polymer Vesicles

by

Allison Hamilton

A Thesis
Presented to the Graduate and Research Committee
of Lehigh University
in Candidacy for the Degree of
Master of Science

in
Mechanical Engineering

Lehigh University
August 15, 2003
This thesis is accepted and approved in partial fulfillment of the requirements for the Master of Science.

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Acknowledgments

I would like to thank my advisors, Professor Delph and Professor Vinci, for all their guidance and support while throughout this project. Your positive attitudes, encouragement, and knowledge made my experience valuable both in my growth as a person and as an engineer. I could not have completed this without you.

Walter Brown, thank you for all of your ideas, time, and knowledge that helped me finish this project. You truly went above and beyond to help me accomplish so much. You are a true inspiration.

Maria Santore, thank you for sponsoring the sensor-response system project. I appreciate the time you gave to show me around your facilities and answer all my questions.

Funding for this work was provided by the National Science Foundation, Division of Chemical and Transport Systems.
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I. ABSTRACT

The vesicle rupturing system that was designed, manufactured and tested for this project is part of a larger program to develop a microfluidic device that will mimic the human immune system. There are two main components of this planned sensor-response device: diblock copolymer vesicles (sacks 5-40 \( \mu \text{m} \) in diameter) and a microfluidic device that amplifies a chemical signal using a process called a “vesicle cascade.” In the cascade process a vesicle is activated by a chemical in the environment and ruptured, releasing its contents. The release of its contents amplifies the response to the original triggering chemical well beyond what would be possible with a single vesicle. The sensor-response system is intended for use in different areas, including biological, pharmaceutical and agricultural sciences.

The vesicle rupturing system is the element of the proposed device that actually ruptures the vesicles. The device was designed with six main components: the perforated plate, the top well, the bottom well, the gaskets, the clamp, and the tubing system. The perforated plate has 100, 10 \( \mu \text{m} \) diameter holes arranged in the center. When a pressure differential is applied across the plate, the vesicles, theoretically, are forced through the perforations. This increases the tension in the vesicle membranes and causes them to rupture.

The device was constructed and tested using distilled water as a test medium. Laser drilling was successful at creating perforated plates that are sufficient for testing purposes. The device meets the requirement of reusability of the main components, and is compatible with the dimensional requirements of an inverted microscope. The pressure system is capable of applying pressure over the full range typically needed for vesicle
rupture. The device successfully contains the test fluid without discernable leaks and fluid flow has been demonstrated and measured. Future work must address several challenges that have arisen, including the production of air bubbles that cause the flow rate to deteriorate when operating in a negative pressure mode, and the tendency for contamination over long periods of time that causes blockage of the flow channels.
II. INTRODUCTION

This effort is part of a larger investigation where the goal is to develop a chemical sensor-response system designed to mimic the human immune system. A block diagram is shown in Figure 2.1 (Santore, 2000).

![Figure 2.1: Schematic of a Sensor-Response System](image)

There are two main components of the sensor-response system: the polymeric vesicles and the vesicle cascade. Polymeric vesicles¹ behave like lymphocytes, more commonly known as the white blood cells of the immune system. Surface receptors located on predefined vesicles (a mixture of vesicles with different surface receptors are present in the solution environment) are activated when small amounts of target compounds are found in the vesicle’s surroundings. This activation of vesicles allows them to be easily separated from the mixture.

The separated, activated vesicles replicate through a cascading process (discussed in more detail in succeeding sections) that broadly models bone marrow.

¹ A small sack or cyst, especially one containing fluid (dictionary.com).
The vesicle’s replication is dependent on a lysis\(^2\) process. By rupturing the membrane, the contents of the vesicle are released. Specialized response vesicles (mimicking plasma cells) are ruptured in the final lysis chamber and flushed back into the original mixture so as to counteract the target compounds that originally activated the vesicles.

The sensor-response system will be a complete system that will run continuously in a cascading fashion. Critical steps will be repeated until the goal is achieved: either when all specialized compounds (initially activating the vesicles) have been counteracted with the content of the vesicles, or when a predetermined amount of rupturing has occurred. The multiple steps will be contained in a compact, chip-scale device in which the sensor and process control features are integrated.

In its application, the sensor-response system will be used in both biological and non-biological settings. In the biological field, the chip-size device will be used as a smart drug delivery system which can respond to its environment, for example, to accurately control hormones or to test for pathogens and release counteracting drugs accordingly. In non-biological settings, the sensor-response system can contribute in helping the syntheses of specialized compounds such as pharmaceuticals or in wet environmental and agricultural applications. In aiding synthesis of pharmaceuticals, the sensor-response system could be used in monitoring the supply of additional reactants and/or counteracting side reactions by cascading vesicles with inhibitors. In

\(^2\) Lysis is a process in which the lysin (the perforated plate in the vesicle rupturing system, or in pipette in the single pipette process, both of which are described in following sections) destroys the vesicle membrane.
the agricultural context, the device could protect crops by releasing fungicides without interfering with the crops, or fight harmful bacteria in water bodies such as fish farms.

The sensor-response system is being developed by researchers at the University of Massachusetts under the direction of Dr. Maria Santore with funding from the National Science Foundation. The polymeric vesicles and vesicle rupturing systems will be discussed in following sections. The vesicle rupturing system, which is the main focus of this project, is the second phase of a multiple-stage development process required to create the vesicle cascade portion of the sensor-response system. The initial phase, the single-pipette process, is already in use and is discussed below.

a. Polymeric Vesicles

Phospholipid vesicles are commonly used in biological applications because of their close relation to biological cells. However, the recent invention of diblock co-polymeric Giant Unilamellar Vesicles (GUV's)\(^3\) has proved more advantageous for this application than the phospholipids. Diblock co-polymeric GUV's have molecularly-thin bilayer membranes that carry drugs and cosmetics by forming a sack around an aqueous solution. The specialized design allows the polymeric GUV's to rapidly release their contents upon rupturing, unlike commonly used vesicles fabricated of latexes or gels.

GUV's are desirable in this application because of their uniformity from vesicle to vesicle. Another advantage is the longevity of the vesicle. The original contents of the vesicles are still contained in the vesicle up to 15 months after

\(^3\) Large vesicles, microns in diameter, that are made of one bilayer.
manufacture. The reason for this long life is the hydrophilic\(^4\) portion of the copolymer—Polyethylene oxide (PEO)\(^5\). When the vesicles form, a polymer brush materializes, yielding a robust and stable vesicle (Santore, 2000). Standard phospholipid vesicles stabilized by hydration forces are weaker than PEO vesicles.

The hydrophobic\(^6\) portion of the co-polymeric membrane is also advantageous. It is an 8 nm thick polymer melt\(^7\) compared to a 3 nm layer of hydrocarbon liquid, the hydrophobic half of phospholipid vesicles. The thicker hydrophobic layer results in water permeation 2 orders of magnitude less than that of the phospholipid vesicles. The polymer melt also contributes to the robust mechanical properties, and yields a strain to rupture of approximately 20-30%, compared to the 3-5% for the standard vesicle. Thus the energy required to burst the vesicle is much greater when using the copolymer GUV’s.

Researchers at both the University of Pennsylvania and Lehigh University have tested these polymeric vesicles under extreme conditions and have observed promising results. Copolymer GUV’s are able to withstand both high temperatures (5-10% remained intact at 100 C) and pH’s ranging from 2-11 (Santore, 2000). They have proven to be undetectable by white blood cells, and promising results have been shown in other biocompatibility studies.

---

\(^4\) The portion of the copolymer that is attracted to water. ([http://www.cbc.umn.edu/~mwd/cell_www/chapter2/membrane.html#HYDRO](http://www.cbc.umn.edu/~mwd/cell_www/chapter2/membrane.html#HYDRO))

\(^5\) PEO corona, which creates the exterior, is a protein-resistant material which contributes to the biocompatible nature of the vesicle. ([http://www.cbc.umn.edu/~mwd/cell_www/chapter2/membrane.html#HYDRO](http://www.cbc.umn.edu/~mwd/cell_www/chapter2/membrane.html#HYDRO))

\(^6\) Regions of the copolymer repelled from water. ([http://www.cbc.umn.edu/~mwd/cell_www/chapter2/membrane.html#HYDRO](http://www.cbc.umn.edu/~mwd/cell_www/chapter2/membrane.html#HYDRO))

\(^7\) A bulk polymer phase as opposed to a solution.
The copolymer GUV's are currently filled with a 0.25 molar (340 molecular weight) sucrose solution for testing purposes. The surrounding environment, when the rupturing process occurs, is a 0.25 (260 molecular weight) molar glucose solution. Transport of the solution through the vesicle membrane occurs until the osmotic pressures of the sucrose and glucose solutions equilibrate, yielding a spherical-shaped vesicle. This equilibrium state is required for the rupturing process to occur. If the osmotic pressures are not equal, the vesicle can be over inflated or under inflated, causing difficulty in the lysis process. The vesicle size ranges from 5-40 μm.

b. Vesicle Cascade

The vesicle cascade is the second component of the sensor-response system, and the process that amplifies certain selected, activated vesicles. The vesicle rupturing system itself is a critical step in developing the vesicle cascade. The cascade is analogous to that of a photomultiplier tube (PMT). In a PMT, an initial photon impinges upon a photovoltaic cell. The cell then releases an initial set of electrons. These electrons enter a series of plates that release an additional set of electrons for each initial electron that enters. This process repeats as each additional plate multiplies the total number of electrons.

While holding to the same basic principles as the PMT, the vesicle cascade is more complex. The polymeric vesicles enter the activation chamber, as shown in Figure 2.1, and mix with a stream that contains the target compound. The target compound attaches to the surface receptors on certain vesicles. The number of activated vesicles is proportional to the amount of target compound in the
environment. Separation then occurs in which the non-activated vesicles are recycled, while the activated vesicles are guided to the lysis chamber. The rupturing of the activated vesicles releases a chosen compound. This mixture is then pushed back into the activation chamber and the previously recycled vesicles have the opportunity to be activated. This process continues until a prescribed concentration has been generated. The amplified solution is then released into the desired environment, for instance, the body in biomedical applications.

The unique feature of the sensor-response system is that the final solution can contain vesicles that differ from the initial vesicles. For example, consider a four stage process used to combat a certain bacterium. The vesicles in the initial three stages could sense that pathogen; however, in the fourth stage when the prescribed amplification has been reached, the lysis process would rupture the vesicles containing the counteracting drug.

The goal of the vesicle cascade, and thus of the vesicle rupturing system, is to achieve the maximum amplification possible to rupture all of the activated vesicles from the previous stage.

c. Single-pipette process

In creating the vesicle cascade, the first developmental phase is the single pipette rupturing process. In this process, the vesicle’s membrane is broken by attaching the vesicle to one end of a glass pipette and creating a negative pressure change through the opposite end, thus bursting the vesicle’s membrane. This process is called sieving. The action occurring can be illustrated by imagining a water balloon
being sucked through a small hole. A block diagram of the sieving process can be seen in Figure 2.2 (Santore, 2000). Using the single pipette process only allows a single vesicle to be rupture at a given time.

Figure 2.2: Block Diagram of Sieving Process (Before and After Lysis)

The single-pipette process begins by heating and drawing a glass pipette using a machine called a Needle Pipette Puller. The Needle Pipette Puller draws a standard glass pipette to a fine point, sealing the end. This step is required to decrease the diameter of the original pipette. In order to reopen the sealed end, the fine point is removed by melting a glass bead onto the point. This bead is then cracked off and an open tube forms. The drawn end, the diameter of which is 5-10 μm, is then inserted into a well of vesicles which is located on an inverted Nikon Diphot 300 florescence microscope. This is shown in Figure 2.3 below.
Figure 2.3: Drawn End of Glass Pipette Inserted in Well

A fine tube is attached to the opposite end of the glass pipette. This tube provides the pressure change required to rupture the vesicles. The pressure change is achieved by creating a water manometer. A syringe is attached to the top of the manometer which, when retracted, yields an increase in the volume of air, V. The number of moles, N, along with the gas constant, R, and the temperature, T, remain constant. Ideal gas laws, shown in equation 2.1, yield the theoretical pressure change that occurs as the syringe is extended and the volume change occurs. If the initial state is $P_I$ and $V_I$, the volume increases in state 2, therefore causing the pressure in state 2 to decrease. This creates the pressure differential.

$$PV = NRT \quad \text{or} \quad P_I V_I = P_2 V_2$$  \hspace{1cm} 2.1

The vesicle completely obstructs the opening of the drawn end of the glass pipette, preventing flow through the system. Therefore, ideal gas laws can be utilized to calculate the pressure change, as the only change occurring in the system is the
A fine tube is attached to the opposite end of the glass pipette. This tube provides the pressure change required to rupture the vesicles. The pressure change is achieved by creating a water manometer. A syringe is attached to the top of the manometer which, when retracted, yields an increase in the volume of air, \( V \). The number of moles, \( N \), along with the gas constant, \( R \), and the temperature, \( T \), remain constant. Ideal gas laws, shown in equation 2.1, yield the theoretical pressure change that occurs as the syringe is extended and the volume change occurs. If the initial state is \( P_1 \) and \( V_1 \), the volume increases in state 2, therefore causing the pressure in state 2 to decrease. This creates the pressure differential.

\[
P V = N R T \quad \text{or} \quad P_1 V_1 = P_2 V_2 \quad 2.1
\]

The vesicle completely obstructs the opening of the drawn end of the glass pipette, preventing flow through the system. Therefore, ideal gas laws can be utilized to calculate the pressure change, as the only change occurring in the system is the
increase in volume of air with the resulting pressure change. Figure 2.4 is a block diagram of the system and components involved in application of pressure through the pipette. During physical experimentation, the pressure is displayed through the use of a pressure transducer.

![Figure 2.4: Block Diagram of the Single-Pipette System](image)

The change in pressure through the glass pipette forces the vesicle through the drawn opening, thus increasing the tension in the vesicle membrane until the maximum tensile strength is exceeded and the membrane ruptures. This relation is shown through equation 2.2 below (Santore, 2000).

$$\tau = \frac{\Delta P \cdot R_p}{2 \left(1 - \frac{R_p}{R_o}\right)}$$

\(\tau\) = Membrane Tension  
\(\Delta P\) = Change in Pressure  
\(R_p\) = Radius of Pipette  
\(R_o\) = Radius of Vesicle

---

*Vesicle must be considerably larger than the drawn end of the pipette to create the tension required.*
In order for maximum pressure change to occur, the vesicle must completely block the drawn end of the pipette. The chemical makeup of the vesicle allows adhesion to occur, forming a seal around the opening of the pipette. This adhesion property of the vesicle is also critical to bursting the vesicle, because it holds the vesicle in place while the surface tension of the membrane is increased.
III. FINAL DESIGN

The main purpose of the vesicle rupturing system designed and constructed in this project is to increase the capabilities of the single-pipette process by rupturing more vesicles at a given time. This is critical for the creation of the vesicle cascade. The single glass pipette has been replaced by a perforated glass plate with two stainless steel wells located on either side used as fluid chambers. This is illustrated in Figure 3.1.

![Cross Section Block Diagram of Lysis Process](image)

**Figure 3.1: Cross Section Block Diagram of Lysis Process**

The pressure change occurs across the glass plate, which is perforated with one hundred 10 μm diameter channels. These channels, theoretically, behave like one hundred of the drawn ends of the glass pipettes described in the previous section. The pressure change and governing equations, in theory, stay consistent with the vesicle rupturing system. Therefore, they were used as guides in the design process.
The final design consists of six main components: the perforated plate, clamp, the bottom well, the top well, the gaskets, and the tubing system. An exploded view of the system is shown below in Figure 3.2. All mechanical drawings have been created using I-DEAS version 10 software package (Electronic Data Systems Corporation).

Some components had a unique design criterion determined by the researchers at the University of Massachusetts, while other specifications were indirectly linked through relating pieces. The overall system had four important design requirements: (1) the vesicles must stay within a 2 mm vertical distance from the base of the device before, during, and after the rupturing process to allow it to be viewed by a 20 x objective on the Nikon Diphot 300 microscope at the University of Massachusetts; (2) the device must be transparent in the areas that are required for viewing before, during, and after the rupturing process; (3) the device must be able to be cleaned to remove residue; and (4) the device must be reusable. Table III.I describes the parts and their functions. The middle stack, which is referred to later in this document, consists of the top well, the gaskets, the perforated plate, and the bottom well. Detailed requirements for each component, along with the design options explored, are described in the following sections.
Figure 3.2: Exploded Isometric View of the Final Design

Table III.I: Exploded View Description

<table>
<thead>
<tr>
<th>Name</th>
<th>Outer dimensions (mm)</th>
<th>Material</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>A Top Well</td>
<td>25 x 25 x 0.635</td>
<td>Stainless Steel</td>
<td>Hold vesicle-containing fluid before rupturing</td>
</tr>
<tr>
<td>B Gasket</td>
<td>25 x 25 x 0.152</td>
<td>Latex</td>
<td>Provide seal</td>
</tr>
<tr>
<td>C Perforated Plate</td>
<td>25 x 25 x 0.1</td>
<td>Glass</td>
<td>Rupture vesicles</td>
</tr>
<tr>
<td>D Gasket</td>
<td>25 x 25 x 0.152</td>
<td>Latex</td>
<td>Provide seal</td>
</tr>
<tr>
<td>E Bottom Well</td>
<td>25 x 25 x 0.122</td>
<td>Stainless Steel</td>
<td>Hold fluid after rupturing</td>
</tr>
<tr>
<td>F Tube</td>
<td>0.812 Diameter</td>
<td>Stainless Steel</td>
<td>Connection to tubing system</td>
</tr>
<tr>
<td>G Clamp Top</td>
<td>74.1 x 40.8 x</td>
<td>Aluminum</td>
<td>Provide clamping force</td>
</tr>
<tr>
<td>H Clamp Base</td>
<td>74.1 x 40.8 x</td>
<td>Aluminum</td>
<td>Provide clamping force</td>
</tr>
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</table>
a. Perforated Plate (C)

i. Preliminary Research

The perforated plate is the main component responsible for rupturing the polymer membrane of the vesicles. It is also the most vital and constrained piece of the overall design. When a pressure difference is created across the plate, the vesicles will be pulled towards the holes and forced through the perforations. The forcing process will cause the vesicles to rupture, releasing the contents through the backside of the plate in the direction of the decreasing pressure. The chemical characteristics of the fluid, along with the tools utilized by the researchers at the University of Massachusetts, provided the design criteria for the plate. First, the plate must be made of a transparent material so as to allow for viewing of the suspended vesicles before, during, and after the rupturing process through the use of an inverted microscope. The material must also have enough stiffness to resist extreme deformation under the expected pressure difference across the plate. Glass is the preferred material because it is chemically inert and easy to clean.

The holes perforating the plate should be 5 to 10 μm in diameter. This range matches the current, single pipette process, as described in the Introduction section. The number of holes desired is, however, unspecified. At least 50 μm of plate thickness is necessary for the vesicle to completely adhere to the edge and the inner surface of the hole and rupture during the pressure application. This thickness should be sufficient to prevent the vesicle from squeezing through the perforation without bursting.
This high aspect ratio of 10:1 (thickness to hole diameter) rules out many conventional microscale manufacturing processes, for instance, photolithography and etching. Therefore, specialized methods were researched. Initially promising ways of achieving the requirements were determined to be cryogenic etching, etching using Foturan glass, the use of microchannel plates and microfilters, and laser drilling.

Cryogenic etching with a reactive gas greatly enhances the anisotropy of conventional etching dry processes by increasing the perpendicularity of the through hole walls—a process that provides for a small amount of taper (Kovacs, 1998). Achievement of cryogenic etching requires the substrate to be cooled by helium gas to liquid nitrogen temperatures, approximately -195 °C. This process is able to yield aspect ratios of up to 30:1 (pure SF₆). It works for many materials, including silicon, but not for silicon dioxide. A thin, outside layer of a silicon substrate could be oxidized to produce an outer layer of silicon dioxide. However, silicon does not satisfy the transparency requirement that is essential for viewing the rupturing process of the vesicles and therefore needs no further consideration.

Foturan glass is a photosensitive glass material containing small amounts of noble metals (Lui and Vasile, 1999) that allows for etching of fine structures while maintaining standard glass properties such as strength, hardness, and chemical resistance. Created by Mikroglas Corporation, this unique type of glass allows for minimum feature sizes of 25 μm in diameter with high repeatability and roughness of 1 μm. Due to the anisotropic nature of the material, an aspect ratio of 20:1 is easily achievable. Foturan sheets can be manufactured as thin as 150 μm.
In order to accommodate the shortcomings of Foturan glass (minimum through hole diameter of 25 μm and surface roughness of 1 μm) spin-on glass could be utilized. Spin-on glass (SOG), most commonly used in manufacturing integrated circuits, is a high quality dielectric material that is a mixture of SiO₂, a solvent, and dopants such as phosphorus or boron (Lui and Vasile, 1999). SOG is applied to the substrate in liquid form and spun, creating a thin layer, similar to the application of photoresist. Coating the patterned Foturan with multiple coats of SOG would thus be likely to decrease the diameter of the perforations and produce a finer surface roughness, thus meeting the requirements of the perforated plate.

The main disadvantage of Foturan is the expense of the process. Foturan requires a laser written, chrome-on-quartz mask that would cost approximately $1,500. An additional $5,000 for processing and manufacturing would be required to produce 20 plates. Since the price per piece would decrease as the quantity increased and the cost of the mask would be spread over a larger number of plates, Foturan would be a viable option for the large scale manufacturing stage, but not in the prototype and developmental stage.

Prefabricated structures such as microchannel plates and microfilters, or modifications thereof, were also researched as potential possibilities for the perforated plate. Microchannel plates are commonly used as secondary-electron multipliers, deflecting and amplifying electrons. The main component of the microchannel plate is a leaded-glass disk, called a capillary plate, which contains thousands of tiny channels (diameters ranging from 6-25 μm). The thickness of the glass plate varies, but the typical range is between 0.25-1 mm (hama-comp.com). This range of dimensions fits
the criteria set by the researchers at the University of Massachusetts. The problem associated with the use of a standard microchannel plate is the open area ratio of approximately 0.6. As the open area ratio increases, the change in pressure that occurs across the plate decreases. This is illustrated through equations 3.1 and 3.2 that are determined using fully-developed, laminar, incompressible, viscous channel flow with a constant pressure change, and the conservation of mass principle (Fox & McDonald, 1998). These calculations are based on a preliminary system determined in the preliminary research and development stage with "best guess" values.

\[ R = \frac{n \cdot A_{\text{channel}}}{A_p} = \frac{n \cdot R_{\text{channel}}}{R_p^2} \quad \text{(3.1)} \]

\[ \Delta P = \frac{8 \cdot V_p \cdot R_p^2 \cdot L_{\text{channel}} \cdot \mu}{R_{\text{channel}}^4 \cdot n} = \frac{8 \cdot V_p \cdot L_{\text{channel}} \cdot \mu}{R_{\text{channel}}^2 R} \quad \text{(3.2)} \]

- \( R \) = Open area ratio
- \( V_p \) = Velocity of piston
- \( A_p \) = Cross-sectional area of piston
- \( R_p \) = Radius of piston
- \( L_{\text{channel}} \) = Length of channel
- \( R_{\text{channel}} \) = Radius of channel
- \( A_{\text{channel}} \) = Cross-sectional area of channel
- \( \mu \) = Viscosity
- \( n \) = Number of channels

The open area ratio is directly proportional to the number of channels in the perforated plate, as shown in equation 3.1. Therefore, a high open area ratio is proportional to a large number of channels, \( A_{\text{channel}} \) and \( A_p \) are fixed. As \( n \) increases with all other variables held fixed, the pressure (equation 3.2) decreases. Using the equations 3.1 and 3.2, the relationship shown in Figure 3.3 is derived.
Figure 3.3: Change is Pressure vs. Open Area Ratio

The pressure change that occurs across the plate is the only mechanism for rupturing the vesicles. Therefore it is critical to the design of the system. Through previous experimentation at the University of Massachusetts, a pressure differential of approximately 1-10 kPa is required during the lysis process. Consequently, in standard form with an open ratio of approximately 0.6, microchannel plates cannot produce the desired pressure differential.

Microfilters, commonly fabricated by Pall Corporation, are used in a wide variety of applications such as the medical and semiconductor industries, as well as in pharmaceutical sciences and contamination prevention in fluid systems. They have the same drawback, however, as the microchannel plates: a large open area ratio. Custom manufacturing modifications of either device lead to extremely high costs—
again uneconomical at the prototype stage. The estimated cost from Hamamatsu, a large manufacturer of microchannel plates, for custom modifications was approximately $800 per plate, thus confirming microchannel plates as an unviable option.

Another feasible option is laser drilling. Commonly used for micro hole drilling, an Excimer laser can attain feature sizes as small as 5 μm with aspect ratios up to 100:1 (Resonetics.com). However, a slight taper of the walls within the feature (through hole in this application) is prevalent, yielding a slight decrease in size from the front surface of the plate to the back. Possible materials include plastics, polymers, ceramics, and metal; lasing glass is not a problem. As a one-step alternative to chemical etching and other traditional methods of micro manufacturing, laser drilling provides an economical option for manufacturing the perforated plate. Through Cornell University’s Nanobiotechnology Center, an Excimer laser is available at the cost of $35 per hour. Since laser drilling met the requirements set forth and is economically feasible, this option was further explored.

ii. Laser Drilling

A laser is a device that generates and intensifies light. Three critical elements comprise a laser system: (1) the laser medium (gas, liquid, or solid); (2) the pumping process; and (3) the optical feedback element (Resonetics.com). One of the advantages of using lasers in material processing is that it is a non-contact device, thus eliminating complications due to tool wear, a problem commonly encountered in traditional drilling. Moreover there are no chemical solvents, thus reducing the waste.
handling costs and leading to an environmentally clean process alternative. It is also appropriate for prototype work due to the negligible tool-up costs (Resonetics.com).

Lasers are categorized according to the medium used to generate the beam. Four different media can be used: gas, solid state, semiconductor, and liquid dye. Gas and solid state are the two media appropriate in material processing. Within these two classifications, there are three main types of lasers. They are Carbon Dioxide, Solid State ND\textsuperscript{3}, and Excimer lasers (Resonetics.com). Each type of laser possesses unique properties making them useful for different applications.

CO\textsubscript{2} lasers are common in the industry because they are inexpensive. Carbon Dioxide lasers have a practical minimum feature size of 200 \( \mu m \) (Resonetics.com). Solid State ND\textsuperscript{3} utilize the doping properties of rare earth metals to combine to form the host material or medium. A readily obtainable minimum feature size using a Solid State laser is 50 \( \mu m \) (Resonetics.com). Machining with CO\textsubscript{2} or ND\textsuperscript{3} is a thermal process in which the laser overloads the target surface with heat and vaporizes the material.

The third type of laser is an Excimer laser. Noble gases comprise the medium used in an Excimer laser (Resonetics.com). In the ground state, noble gases do not readily form compounds. However, though excitation, noble gases react with certain elements and form compounds referred to as “dimers.” These compounds, the dimers, release UV emissions that perform the drilling process. The frequency of Excimer UV emission is extremely high and breaks the bonds of the material which is being lased (Resonetics.com). Therefore, with Excimer lasers, machining occurs through ablation instead of through thermal overload as with Carbon Dioxide and Solid State lasers.
Ablation leaves a cleaner, smoother surface than burning and vaporization, which is more desirable in our application. With a practical resolution limit of 5 μm, which is less than the required channel diameter for this project, an Excimer laser was the best choice for manufacturing the perforated plate.

A mechanical drawing of the front view of the perforated plate is shown below in Figure 3.4. A complete set of mechanical drawings can be found in Appendix A.

![2 x 2 mm matrix of 10 μm diameter holes](image)

**Figure 3.4: Front View of Perforated Plate**

The plate is a 2.5 x 2.5 cm glass square with a 2 mm matrix of 100 holes with 10 μm diameter equally spaced in the center. The glass plate is 0.1 mm thick. The number of holes was chosen by evaluating the time required to produce the holes (around 10 min per hole) and the open area pressure calculations shown in Equations 3.1 and 3.2. One hundred 10 μm diameter holes with an exposed surface area of 7 mm² lead to a very small open area ratio which allows for an ample pressure
differential to occur across the plate. The 2 x 2 mm square within which the holes are located is centered between the 3 mm cavities of the bottom and top well, so that slight misalignment of the stacked component would not cover any holes.

Preliminary efforts to produce the perforated plate by laser drilling were undertaken using the Excimer laser at Cornell University's Nanobiotechnology Center. However, these preliminary efforts yielded undesirable results. After numerous attempts, it was concluded that the Cornell laser did not have the energy output or the precise focusing capabilities required to produce the 10 μm diameter holes that were desired. Resonetics has a more precise and powerful laser and was contracted to drill the 10 μm diameter holes.

### iii. Results

The holes drilled by Resonetics vary in shape and size. This can be seen below in Figures 3.5 thru 3.10 that were taken in a JEOL 6300 Scanning Electron Microscope. As shown through the pictures, the front side of the holes, where the laser enters, has a round shape and measures between 20-30 μm in diameter. This oversized front hole is required to drill a 10 μm hole on the backside, due to the inherent taper that occurs when drilling the small holes. Figures 3.5 thru 3.10 show the backsides of randomly selected holes and also show the inconsistency in the shape. Resonetics is unable to explain the variations in the shape. Although the holes are not perfectly shaped, they fit the 5-10 μm diameter range required by the project and are used for this development stage.
Figure 3.5: Front, Four Holes

Figure 3.6: Front, Up-close

Figure 3.7: Back, Four Holes

Figure 3.8: Back, Up-close

Figure 3.9: Back, Up-close

Figure 3.10: Back, Up-close
b. Bottom Well (E)

The purpose of the bottom well is to collect the fluid after the rupturing process has occurred. It is also the piece that provides the connection from the tubing system to the assembled design, yielding the ability to apply a pressure change to the system. The bottom well is a 2.5 x 2.5 cm square piece that is 0.122 cm thick. This thickness was chosen to yield to the 2 mm height limit determined by the microscope objective. In the front view, there is a 3 mm hole in the center that extends through the thickness of the piece. This feature holds the liquid after the vesicles have been ruptured and allows for viewing of the rupturing process. The 3 mm hole lines up with the 2 mm hole matrix of the glass plate and the 3 mm hole that extends through the top well in the same manner. From the side, a 0.89 mm hole extends from the outer edge of the well to the 3 mm hole that is bored through the center of the piece. The intersection of the 3 mm hole and the 0.89 mm hole forms a 90° angle. AISI type 304 stainless steel was chosen for the material of the well because of its mechanical strength, along with its low reactivity. A 0.25 mm thin glass disk is sealed to the underside of the bottom well covering one side of the 3 mm hole, completing the fluid chamber. The glass disk is 15 mm in diameter and satisfies the transparency requirement, allowing viewing through the 3 mm center hole. An isometric mechanical drawing of the bottom well can be seen below in Figure 3.11. The complete mechanical drawing can be seen in Appendix A.
Figure 3.11: Isometric Mechanical Drawing of Well

The well was manufactured by V & M Tool Company in Perkasie, PA using a combination of wire and ram Electrical Discharge Machining (EDM) techniques. EDM, in general, uses an electrode with timed electrical pulses to evaporate the material. Both the work piece and the electrode are held by the machine and controlled through an automated controller. The work piece remains stationary while the electrode moves to create the specified design. A power supply is responsible for releasing the electrical discharge in varying but predefined time and intensity levels.

The discharge of electricity begins where the electric field is the strongest. Electrons and positive free ions are accelerated to high velocities from the electric field to produce an ion channel. The ion channel is the conductor of electricity. At this point, the current is able to flow from the diode to the work piece. Upon contact, a spark forms between the work piece and the electrode and a bubble of gas develops. The bubble's pressure and temperature rises, causing a plasma region to develop having
extremely high temperatures in the range of 8,000-12,000 C (mmsonling.com). When
the electricity is turned off, the drastic decrease in temperature causes the bubble to
implode, thus projecting the material away from the work piece and leaving a crater in
the material. This process is repeated numerous times, creating the cutting process.

The outer edges and the centered 3 mm hole are cut using wire EDM. Wire
EDM uses an electrical wire, as the electrode, held in position on both ends by the
machine, like dental floss held between two hands. Both ends of the wire move
simultaneously, cutting the material in contact to the intended dimensions. In order to
use this technique for internal features, an entrance hole must be drilled using, most
commonly, conventional methods. This entrance hole allows the wire to be threaded
through the piece so that the boundaries of the feature can be traced out.

Ram EDM was used to create the 0.89 mm diameter hole through the side of
the piece. Due to the 0.813 mm diameter hypodermic stainless steel tubing that had to
be inserted through the length of this hole, the round and straight nature of this hole is
crucial to ensure the tube will not be damaged when inserted. Another difficulty with
this feature is the clearance material on the side of the hole. Only 0.039 mm of
material will remain on each side of the hole. It is because of this feature that the well
cannot be manufactured using conventional machining techniques. Therefore, ram
EDM was chosen as the best alternative because this technique is reliable and stable.
Ram EDM uses an electrode in the shape of the desired feature, in this case a tube, to
perform the cutting process. A force is applied to the electrode forcing, or ramming,
through the work piece, which is held stationary to cut the intended shape.
Approximately 3 minutes of process time of ram EDM is required to produce the 0.89 mm hole in the well.

c. **Clamp (G and H)**

The clamp serves two main functions for the vesicle rupturing system. First, it provides the compression force required to produce a leak free design. Second, it is the frame of the system which allows the system to easily sit on the inverted microscope. The clamp has two main pieces, the top and the bottom. Four 6-32 screws attach the top and the bottom pieces with the middle stack located between them (the location can be seen in Figure 3.2 from the design introduction). When the screws are tightened, the stack is compressed, forming the seal. Figure 3.12 and 3.13 show isometric views of the top and bottom pieces, respectively. A complete set of mechanical drawings for both pieces are shown in Appendix A.
Four holes to pass 6-32 screws

2 cm diameter hole for access to top well

Figure 3.12: Isometric View of Top Clamp

Four threaded holes to fit 6-32 screws

2 cm diameter hole for glass slide

2.6 x 2.6 x 0.4 cm square

3.5 mm diameter hole for tubing connection

Figure 3.13: Isometric View of Bottom Clamp
The top component of the clamp is made from a rectangular 4 x 7 x 0.425 cm piece of aluminum. A 2 cm diameter hole is located in the center that provides easy access to the top well, where fluid containing the vesicles is initially inserted. Four through holes, equally spaced on either side of the center hole, are drilled to pass 6-32 screws.

The bottom component of the clamp is also made from a 4 x 7 x 0.425 cm rectangular aluminum plate. A 2.6 x 2.6 x 0.4 cm square is cut from the center in which the middle stack fits. A 2 cm diameter circle is cut the rest of the way through the aluminum plate in the center to the square. This is to fit the 0.25 mm thick glass slide that is attached to the bottom of the well. A 3.5 mm diameter hole is drilled into the side of the plate, allowing for the attachment of the tubing system. Two threaded holes to fit 6-32 screws are placed on each side of the square.

d. Gaskets (B and D)

Two gaskets are required for sealing the system so that no air or water enters or exits the main chamber. One gasket is located between the bottom well and the underside of the perforated plate, and the other forms the seal between the top well and the top of the perforated plate. The gaskets are produced out of 0.152 mm latex sheeting. They are 2.5 x 2.5 cm square pieces with a 3 mm hole through the center that lines up with the 3 mm holes located in the bottom and top wells. The gaskets and locations can be seen in Figure 3.2: Exploded Isometric View of the Final Design in the design introduction.
In order to drill the center 3 mm diameter holes in the gaskets, the material was cooled to liquid nitrogen temperatures between two flat boards. This method allowed for a clean hole to be created without damaging the surrounding material. When drilled at room temperature, the latex pulls and rips, not providing the desired clean, round 3 mm hole. Other conventional methods, such as punching and cutting, also proved ineffective due to elastic properties of the latex, either sealing the punch together or not fully cutting out the center hole. After drilling the center holes of the latex, a 2.5 x 2.5 cm square was measured and cut out, thus completing the gaskets.

e. Top Well

The top well’s function is to hold the fluid before the vesicles are ruptured. It is located above the top gasket which sits above the perforated plate. The dimensions of the well are 2.5 x 2.5 cm, which allows its outer edges to line up with the bottom well and the perforated plate. There is a 3 mm hole drilled through the well’s center that acts as a fluid chamber. This feature exposes the 2 x 2 mm matrix of holes on the perforated plate. The top well is made out of 0.635 cm thick AISI type 303 stainless steel.

f. Tubing System

The tubing system is responsible for the pressure differential applied across the perforated plate used to rupture the vesicles. It connects to the main system through a 0.812 mm stainless steel tube that is rigidly attached, using epoxy, to the 0.89 mm hole in the side of the bottom well. This connection tube is illustrated in Figure 3.2 as
piece F. An overhead, block schematic of the tubing system can be seen below in Figure 3.14.

![Block Schematic of Tubing System](image)

**Figure 3.14: Block Schematic of Tubing System**

Two main sections branch off from this tube through the use of a "Y" connector. The right side, as shown in Figure 3.14, leads to a pressure transducer. The left side leads to a water manometer that mimics the manometer described in the Single Pipette Process section above. Reducing unions, stopcocks, and Luer lock connectors are employed to connect the various tubing sizes and materials into a leak free system.
The water manometer acts as an amplifier for the pressure change in the system. If the manometer is not in place and the syringe pulls the fluid directly, for a 6 cm$^3$ syringe capacity, the result would be a displacement of 6 cm$^3$ of the fluid. Since the tube being used for this experiment is 0.476 cm in diameter, it would only allow for a pressure change of 8.4 cm of water. This is below that required to rupture a polymeric vesicle (10-100 cm of water). The water manometer, like the one described in the Single Pipette Process, follows the theory used in the ideal gas law, shown below in equation 3.3.

$$PV = NRT \quad \text{or} \quad P_1V_1 = P_2V_2$$

3.3

State one is the initial position when the syringe is compressed. State two is after the syringe has been retracted. The volume in state two, $V_2$, has increased requiring the pressure in state two, $P_2$, to decrease. Preliminary tests using just the water manometer were completed to confirm the system was working as intended. The results are shown below in Figure 3.15 and Table III.II which confirm that the manometer is acting like an amplifier. The initial amount of air in the system determines the pressure change that is caused by pulling the 6 cm$^3$ syringe. As can be seen, a pressure change of 16.5 cm of water has been observed when extending the syringe once.
Figure 3.15: Pressure Comparison

Table III.II: Manometer Results

<table>
<thead>
<tr>
<th>Initial Volume of Air</th>
<th>Trial</th>
<th>Δ Water Height</th>
<th>Average Δ Water height</th>
<th>Δ Volume</th>
<th>Theoretical Pressure (Pa)</th>
<th>Experimental Pressure (Pa)</th>
<th>Error %</th>
</tr>
</thead>
<tbody>
<tr>
<td>190</td>
<td>1</td>
<td>16.1</td>
<td>16.6</td>
<td>3.1</td>
<td>1678</td>
<td>1625</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>16.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>13.1</td>
<td>13.4</td>
<td>3.6</td>
<td>1266</td>
<td>1311</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>13.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>483</td>
<td>1</td>
<td>9.8</td>
<td>10.0</td>
<td>4.2</td>
<td>920</td>
<td>981</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10.4</td>
<td></td>
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<td>9.8</td>
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<td>7.6</td>
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<td>4.7</td>
<td>755</td>
<td>742</td>
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<td>7.5</td>
<td></td>
<td></td>
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</tr>
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<td></td>
<td>3</td>
<td>7.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
The manometer was sized using the ideal gas law shown in equation 3.3. The range of pressures desired by the University of Massachusetts is 10-100 cm of water. Using a 6 cm$^3$ syringe, the initial volume of air required to produce the maximum and minimum pressure, with a full retraction of the syringe, is calculated below.

\[
\frac{P_1}{P_2} = \frac{V_2}{V_1}
\]

3.4

\[
\frac{P_1}{P_1 - \Delta P} = \frac{V_1 + \Delta V}{V_1}
\]

3.5

\[
\left(1 - \frac{\Delta P}{P_1}\right)^{-1} = \left(1 + \frac{\Delta V}{V_1}\right)
\]

3.6

\[
1 + \varepsilon = 1 + \frac{\Delta V}{V_1}
\]

3.7

\[
V_1 = \frac{\Delta V}{\varepsilon}
\]

3.8

$P_1$ = Pressure at state 1 (atmospheric pressure)
$P_2$ = Pressure at state 2
$V_1$ = Volume at state 1
$V_2$ = Volume at state 2
$\Delta P$ = Change in pressure
$\Delta V$ = Change in Volume
$\varepsilon = \Delta P/P$

Equation 3.7 was determined using the first term in a binomial expansion because of the extremely small value of $\varepsilon$. The calculations determine that for a 6 cm$^3$ syringe, $\varepsilon$ for a 10 and 100 cm of water is 0.0097 and 0.097 respectively. This lead to an initial volume of air required of 619 and 61.9 cm$^3$ for the pressures of 10 and 36.
100 cm of water, respectively. From these calculations a standard 1000 cm$^3$ volume was chosen for the manometer. Originally, a plastic Nalgene bottle was used. However, at high pressures, the bottle collapsed inward limiting the maximum pressure attainable. Therefore, a glass bottle is currently being used for the manometer.

The right side of the tubing system connects to a DP45 Validyne pressure transducer in combination with a CD15 Validyne signal conditioner that is used to accurately measure the pressure differential across the plate. The pressure transducer can measure pressures ranging from 0-140 cm of water, well outside the range of 10-100 cm of water required to rupture the vesicles. Since the top well, which initially houses the fluid, is open to the atmosphere, while the top side of the perforated plate, along with the initial fluid is at atmospheric pressure. The pressure is decreased in the system using the water manometer, causing a pressure differential to occur across the plate. The pressure transducer works in the same manner. One side is open to atmosphere and the other is attached to the tubing system. The change of pressure that occurs across the plate is equal to that of the pressure occurring through the transducer, thus producing an accurate pressure measurement. A voltmeter is attached to the signal conditioner, yielding an easy digital read-out of the pressure changes occurring in the system.
IV. UNSUCCESSFUL DESIGN VARIATIONS

The initial design was similar to the final design, except for the clamp. The middle stack, starting with the top well through the bottom well, was not modified and has stayed consistent through the design changes. The initial design is shown in the isometric, exploded drawing labeled Figure 4.1 below. Letters A thru F correspond to Table III.I from the final design introduction.

![Figure 4.1: Isometric, Exploded Drawing of Initial Design](image)

A base, constructed of 76 x 25 x 0.25 mm thin glass bottom (labeled G) with two thicker 25 x 25 x 1 mm glass side supports (labeled H), was attached to the bottom well as a foundation for the middle stack. Four metal brackets (labeled I) were glued to the side supports, two on each side. Two elastic bands stretched across the stack and hooked to the brackets on the opposite side. The purpose of these bands was
to apply pressure to the top of the middle stack to compress the gaskets enough to produce a leak free system. The problem with this design occurred in the assembly process. The bottom well and side supports were bonded using epoxy from Armstrong Adhesive Products. During the curing process, the epoxy shrank, causing the thin glass slide to bend, bowing the side supports upward. Because of the tension that was created in the glass while curing, when the elastic was attached to the brackets, the thin glass slide immediately cracked at the seams between the bottom well and either side support.

This problem was solved by removing the brackets and wrapping elastic bands in the opposite direction (the shorter dimension), around the stack itself. This allowed the pressure producing bands to be in contact with only the middle stack, which is supported by metal. Although this solved the problem of the glass breaking; it did not produce enough force to completely seal the gaskets. This allowed the system to leak. As a result the stiff metal clamp was designed, as described in Section III:c.
V. RESULTS

a. Negative Pressure Testing

Tests on the system were completed using distilled water to fill the system. Red distilled water, dyed with food coloring, was placed in the top well where the vesicle solution would initially be placed. The die was used to visually determine if water from the top well was flowing through the system. A negative pressure, 100 cm of water below atmospheric, was drawn using the water manometer in an attempt to suck the water through the perforated plate. This experiment was run numerous times, but was only successful once. The one time that the experiment worked as intended, the flow rate across the plate was determined to be approximately 0.8 mm\(^3\)/min. In theory, this value should be closer to 4 mm\(^3\)/min. The theoretical value is determined using equation 5.1 for the volume flow rate for laminar pipe flow (Fox & McDonald).

\[
Q = -\frac{\pi \cdot R^4}{8 \cdot \mu} \left( \frac{\partial P}{\partial x} \right) \tag{5.1}
\]

Where:
- \(Q\) = Volume Flow Rate
- \(R\) = Channel Radius
- \(\mu\) = Viscosity
- \(P\) = Pressure
- \(x\) = Length of Pipe

Subsequent attempts to replicate this initial success were unsuccessful. It is suspected that the cause for the lack of flow in subsequent attempts was out-gassing of water that occurs at pressures below atmospheric. The system was completely flushed with air each time an experiment was run. Tiny bubbles began to appear approximately 15 min after the negative pressure was drawn. The number of bubbles increased with time. In the capillary tubing that comprises a majority of the tubing
system, the air bubbles completely filled the diameter of tube. It is thought that these bubbles of air blocked the connection tube to the bottom well preventing the desired pressure differential from occurring across the plate. As the preliminary testing of the current design indicates, the current design does not pass water through the plate consistently. Therefore, rupturing of vesicles cannot take place at this time.

In order to eliminate the out-gassing problem that occurs with drawing negative pressure, positive pressure should be applied to the system to force the fluid through the holes. Preliminary test to prove this hypothesis have been completed and the results are promising.

b. Positive Pressure Testing

In order to achieve the positive pressure, a brass piece was glued to the top well covering the 3 mm fluid chamber. A small hole was drilled through the brass piece to which a piece of capillary tubing was attached. The manometer was removed from the system, and the syringe was directly attached to the to “Y” connector. Figure 5.1 shows a block diagram of the configuration.
Determining the air flow through the modified system was the first experiment performed. Air was the first substance tested because it has a lower viscosity than water, allowing it to flow through the system with greater ease. The system, including the syringe, was filled with air. As the syringe was compressed, air was forced through the stack and out through the capillary tubing. Air flow data was collected three times. The data was collected by counting the number of air bubbles that exited the capillary tubing (placed in a beaker of water) and estimating their size. The results are shown in Figure 5.2. At approximately 100 cm of water, the flow rate of air is 650 mm$^3$/min. The theoretical value calculated using equation 5.1 is approximately 450 mm$^3$/min. The difference in the theoretical and experimental values is due to the estimation of bubble diameter and experimental error.
Figure 5.2: Flow Rate of Air

Next, red colored water was forced into the system using the syringe, and the pressure differential was held manually at 100 cm of water. The red water was observed flowing through the exit capillary at a rate of 0.47 mm³/min. Figure 5.3 below shows the change between length of the water in the exit capillary tube vs. the change in time.
Figure 5.3: Distance vs. Time

From the distance value the volume flow rate was calculated using the inside diameter of the exit capillary tubing (0.762 mm) to determine the displaced volume and dividing by the time. Figure 5.3 shows a fairly linear flow rate (the jump in the graph at 60 min is due to a temporary increase in the pressure). However, there is a slight decrease in the slope observed between 85 and 110 min. This decrease in slope is probably due to a buildup of contaminants along the channel walls in the perforated plate. This conclusion was reached as a result of the decreasing flow of water through the system. Following this initial trial using positive pressure, a subsequent trial indicated a much reduced flow rate. This subsequent trial was performed after the distilled water had been sitting in the system for approximately 12 hrs. This is also an indicator that contaminants present in the distilled water solution partially blocked the capillary channels in the perforated plate. These contaminants could also be causing
the difference in the theoretical and experimental flow rate, 4 mm$^3$/min and 0.47 mm$^3$/min respectively. Another possible source of error is pressure variations.

The difference in the volume flow rates of air and water is due to the viscosities of the two substances. The viscosities of air and water differ by a factor of approximately 100. Also, the buildup of contaminants in the water likely led to the partial blocking of the capillary holes, which would have reduced the flow rate. Therefore, the development of a proper cleaning method will be essential in future work.
VI. FUTURE WORK

a. Proposed Design Modifications

The next design phase should modify the current system so that positive pressure can be used to push the vesicles through the perforated plate, rather than using negative pressure to suck them out. One possible idea is to attach a threaded spacer to the top of the top well around the 3 mm fluid chamber. This would allow the tubing system and the new piece to be screwed on the main system in a leak free manner. Figure 6.1 is a cross-sectional view of the proposed design.

![Figure 6.1: Proposed Future Design](image)

This new design will allow the new piece to be unscrewed from the main stack, the vesicle fluid inserted into the top well, and the tubing system to be reattached. This new design will eliminate the need for the water manometer, as air can be manually pushed into the system through the syringe as the source of pressure.
b. Perforated Plate

Creating the perforations in the perforated plate in a reliable and consistent manner should be a main focus of future work. Laser drilling provided holes that fit within the guidelines set forth for the first round of testing. However, due to the inconsistent shape and size of the hole, a better method would be preferable for succeeding studies. The 10 μm diameter hole is near the low end of the laser’s capability. This could be one reason for the inconsistent size and shape.

Increasing the number of holes through the perforated plate is another aspect to explore. An increased flow rate though the perforations should help to attract the vesicles towards the holes. This will allow a greater number of vesicles to enter (and block) the holes, while still allowing flow to occur.

c. Cleaning of Contaminants

Cleaning the channels of the buildup of contaminants is a critical part of making the system reusable. A proper method of removing the buildup without destroying the perforated plate or increasing the size of the channels should be determined. One possible method would be to use an industrial glass cleaner such as sodium hydroxide to dissolve the buildup of residue.

d. Testing of Vesicles

After water flow experiments are consistently successful, the rupturing of vesicles must be completed. The test vesicles are initially created and housed in a 0.25 molar sucrose solution. For the rupturing tests, vesicles from this sucrose solution will
be diluted with a 0.25 molar glucose solution. The equal molar concentration of the solutions allows the osmotic pressure inside and outside the vesicle to be equal, thus creating a firm, spherical vesicle. The reason for the mixture of glucose and sucrose solutions is due to the fact that sucrose has twice the mass concentration of glucose. This makes the vesicles heavier than the surroundings, causing them to settle and making them easier to find. This mass difference also allows the vesicles to be optically seen using a microscope (the observation of the vesicles is greatly enhanced through the use of a phase contrast microscope lens). For testing purposes, a 20 x microscope objective should be used for viewing the vesicle rupturing.
REFERENCES


I-DEAS vs. 10. Electronic Data Systems Corporation. Plano, Texas. 2003


APPENDIX A:

Mechanical Drawings
TOP WELL (A)
Material: Stainless Steel 303
Scale: x 3
Units: As Marked

TOP

ISOMETRIC

FRONT

RIGHT
BOTTOM WELL (E)

Material: Stainless Steel 304
Scale: x 3
Units: As Marked

.061 cm

1.3 cm

.89 mm

.122 cm

TOP

ISOMETRIC

FRONT

RIGHT
CLAMP TOP (G)
Material: Aluminum
Scale: x 1.5
Units: As Marked

3.7 cm

TOP

ISOMETRIC

Four holes to pass 6-32 screws

5.6 cm

FRONT

RIGHT
CLAMP BOTTOM (H)
Material: Aluminum
Scale: x 1.5
Units: As Marked

Top View

Front View

Right View

Four threaded holes to fit 6-32 screws

Dimensions:
- 3.7 cm
- 7.4 cm
- 7 cm
- 2.6 cm
- 4 cm
- 0.3 cm
- 2 cm
APPENDIX B:

Biography
Allison Hamilton, born in Auburn, Washington on October 29, 1979, lived with her parents, Michael and Donna Hamilton, and sister Amber until she was 18. She moved to Bethlehem, PA in 1998 to pursue an undergraduate degree in Mechanical Engineering at Lehigh University. While attending Lehigh University, she co-oped at IBM in the Microelectronics division as an equipment engineer. She was also involved in numerous extracurricular activities and held offices such as President of Pi Tau Sigma, a national mechanical engineering honor society (2001-2002), and Vice President of the Society of Women Engineers (2001-2002). After graduating in June of 2002 with a Bachelor of Science in Mechanical Engineering with high honors, Allison remained at Lehigh to pursue a Master of Science degree in Mechanical Engineering. Upon completion of her graduate degree, Allison will work for Knolls Atomic Power Laboratory, a division of Lockheed Martin designing nuclear reactors for the US Navy, in Reactor Servicing Operations.
END OF TITLE