UNIVERSITY OF ROCHESTER, BIOMEDICAL ENGINEERING DEPARTMENT

Nanomembrane Array for High-Throughput Drug Screening

Final Design Report

May 4th, 2009

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Introduction

A naturally occurring RNA editing enzyme called APOBEC3G (A3G) has been found to interfere with retroviral infection by introducing C to U deaminations in viral DNA. HIV and other retroviruses possess a viral infectivity factor (Vif) that inhibits the antiviral nature of the A3G protein, targeting the protein for degradation. However, in the absence of Vif, A3G can effectively restrict viral replication.[i]

A3G exists in the body in both a high molecular mass (HMM) and a low molecular mass (LMM) form, with the LMM form found exclusively in peripheral blood. In the LMM form, HIV is unable to produce Vif because the LMM form will mutate the viral DNA before it can be translated, thus inhibiting HIV infection. Candidate drugs are currently being indentified that are able to break HMM A3G found in non-peripheral blood into the LMM form that inhibits HIV infection.[ii] Researchers hope that by inducing expression of the LMM form in both types of blood the body's ability to resist the expansion of HIV will be greatly increased.

Testing a large number of candidate drugs is a daunting task. We are currently developing a device that efficiently integrates an array of porous nanocrystalline silicon (pncSi) membranes with both a dual-arm laboratory automation system and a high performance multi-well plate absorbance/fluorescence reader to be used in a high-throughput testing process. The long-term goal of our design team is to develop a system by which roughly 20,000 candidate drugs can be tested at a rate of about 1,000 drugs per week. While the design of the system has primarily focused on the application to anti-HIV drug testing, it is intended to be universal enough for use in any application where high-throughput protein separation via nano-filtration is desirable given minimal component alterations. The design process has addressed interfaces with existing lab equipment for liquid handling and automation, transfer mechanisms to move candidate drug and solution across the membrane, and analysis of filtrate for LMM hA3G concentration.

Documentation of Problem

Problem Statement

Design a device that incorporates novel pncSi membranes into an array that will sharply separate molecules based on size, typically in the range of 5-25nm. The device should increase the screening capacity for our customer, Dr. Harold Smith, and serve as proof-of-concept for incorporation of a novel silicon membrane material in a true, large scale, high-throughput screening system.

The immediate application of the device will be for high-throughput screening of drug candidates, such as the APOBEC3G screening suggested by our customer, Dr. Harold Smith.

High-Throughput Screening Background

High-Throughput Screening (HTS) is a process by which researchers can perform large numbers of biochemical, pharmacological, or genetic tests in a short amount of time. Often used in drug

discovery testing, HTS allows rapid testing of large compound libraries against one target, looking for a particular desired result. For example, all candidates in a compound library would be tested for their effectiveness at modifying the function of a target protein. During HTS, candidates undergo a first-level screen to identify "hits", or results of interest. In a test of 100,000 to 300,000 candidates, about 100 to 300 hits will be identified, and then tested further during secondary screenings.[iii]

HTS systems commonly interface with devices and systems that help to make the process faster and more efficient. These interfaces include, at a minimum, lab automation robots to perform accurate liquid handling, as well as highly sensitive detection systems to interpret results. During our design process, we will be interfacing with a JANUS Automated Workstation for liquid handling, and a Tecan Microplate Reader for detection. These components will be addressed in greater detail in the discussion of system-level design.

The use of immune assays for molecular specificity is a nearly universal characteristic of HTS systems. However, as a result of their high development costs, intricate biochemical consequences on the molecules of interest, costly reagents, and time requirements during testing, there exists a niche for alternative assay types in HTS. This is the impetus for incorporating pncSi membranes into a HTS array. By acquiring specificity based on molecular size rather than molecular epitopes, we expect to cut down on many of the aforementioned downsides to immune assays.

Nanomembrane Background

The critical aspect of the array being developed is the incorporation of pncSi membranes. The techniques to develop these membranes were discovered at the University of Rochester, and currently there is further research being conducted regarding the preparation and properties associated with the membranes. Current investigations to characterize these membranes have shown great promise in the area of a method for molecular separations.

Conventional methods for the synthesis of membranes used for separations of small molecules involve the precipitation of polymer substrates to form a film to be used as a membrane. Within the film, nodules form which act as a sieve to inhibit the flow of molecules.[iv] However, these membranes are approximately 1,000 times thicker than the molecules that are being passed through them, and are characterized by poor cut off properties, filtrate loss, and low transport rates.[v] Improved flux through these membranes can be achieved by increasing the effective pore size. But, this results in lowering the molecular discrimination of the membrane, already a crucial issue.

Other efforts have been made to develop ultrathin membranes for microfiltration, mostly using nanolithography to pattern the pores or colloidal templates to begin the formation of the membranes. Nanolithography is an expensive technique that is difficult to scale up though and using colloidal templates allows limited control of pore size.[vi] A major issue with these techniques is membrane fouling, which requires such membranes to be run at very low differential pressures. In developing membranes, it is important to pursue characteristics that would result in a low membrane resistance while still maintaining membrane stability. Thus, new membranes should have a pore size greater than the thickness of the membrane, allowing the pores to be shaped as holes rather than as channels, a high

porosity that does not compromise the structural integrity of the membrane, and finally the pore size should not deviate greatly from the maximum pore size.[vii]

The membranes developed at the University of Rochester have been shown to be an ideal solution to the problem of developing a useful microfiltration membrane. These membranes are formed by a multi-step process. The area on a silicon wafer where the membrane is to be placed is patterned using conventional photolithography techniques. Placing oxide layers and then removing them protects a 15nm layer of a-Si on the backside of the silicon wafer. The pores are then made using a rapid thermal annealing phase (30s) rather than by nano-patterning methods. As the silicon crystallizes, nano-pores nucleate and grow, and the size of these pores is determined by the temperature at which the membranes are annealed.[viii] An outline of the method to form the membrane is given in Figure 1. Pore sizes range from approximately 5-25nm, and the thickness of the crystallized membranes is approximately 15nm.[ix] These membranes have proven to be robust, as membranes with a freestanding area of 40,000μm^2 have been shown to withstand a differential pressure of 1atm.[x] Also, flow rates that have been observed are an order of magnitude greater than thicker nanofabricated membranes and greater than nine times faster than conventional dialysis membranes.[xi] One detrimental aspect of these membranes is their somewhat wide pore size distributions. However, the lack of a tail to pore sizes larger than the cut-off size allows membrane specificity and usefulness to remain intact.[xii]

These membranes show much promise in the field of nano-separations as they have overcome many of the current limitations of membranes developed by conventional techniques. It is hoped that the Nanomembrane Array will be the first demonstration of a potential device that will utilize the robust and specific properties of these membranes.

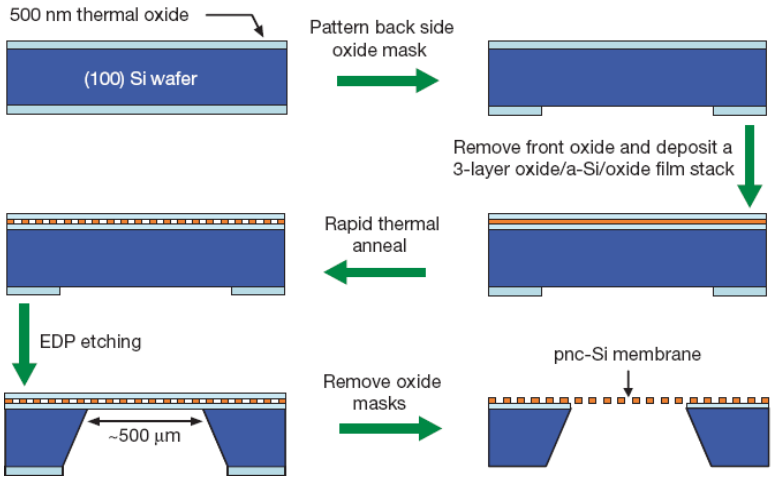


Figure 1[xiii]: An overview of the steps required to develop the pncSi membrane. Note that pore size is largely controlled by changing the temperature of the rapid thermal annealing phase.

Customer Identification

HTS systems are used in applications of all sizes, from large-scale pharmaceutical companies that test libraries of hundreds of thousands of candidates, to smaller-scale testing in a biochemistry research laboratory. Our device is not currently intended for use by a large pharmaceutical company, as they already have complex, high-tech equipment that can perform large screening experiments with minimal user interaction. Rather, our device is intended to be used as part of a high-throughput drug screening system in a biochemistry research laboratory where candidate libraries are on the order of tens of thousands. Although our current prototype device is expected to find immediate use in the area of orthogonal, second-level screening that is used to refine initial hits, we intend future versions of our device to serve as a high-capacity, primary screening device.

Unlike some of the more traditional biomedical engineering devices which cater to caregivers and patients, our team's device accommodates the needs of laboratory supervisors, technicians, and other researchers. While we will deliver our final design to Dr. Harold Smith, a biochemist interested in compounds that disrupt protein complexes involved in HIV, our team is attempting to produce as universally applicable a product as possible. From our standpoint as a "small startup company" we cannot rely on a single customer for all future profits; thus, we have made every effort to design maximal flexibility into our device.

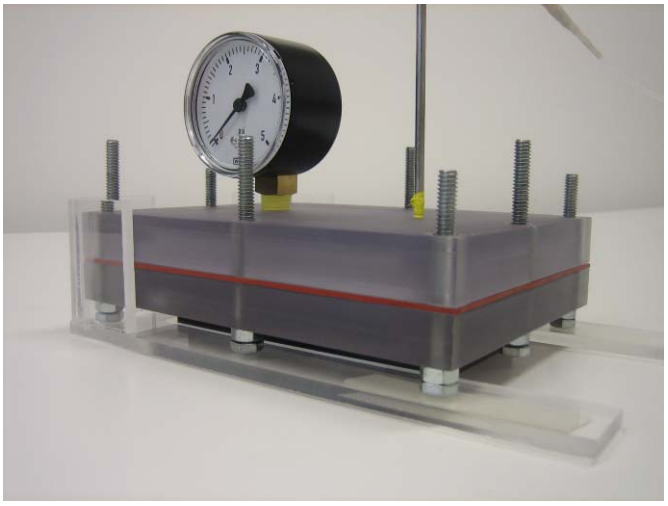
With this in mind, we recognize that certain traits will be universal for all of our target customers. It is expected that our Nanomembrane Array will be used in research laboratories containing common laboratory equipment, such as fluorescence and absorbance detection systems, a compressed air supply (preferably N_2), and technicians to setup and operate the device. The primary use of our device is drug screening by means of molecule separation according to molecular size. Therefore, we anticipate that users will be conducting research or drug development that requires nano-scale filtration at rates greater than those that are currently available through filtration media such as polyacrylamide and agarose gels. Additionally, our design will provide users with the ability to quantify the results of filtration by maintaining compatibility with common laboratory equipment. So, although our device represents an intermediate component in a laboratory's drug screening protocol (between liquid handling and detection), by designing with the entire process in mind we will provide our customer increased screening capacity.

Needs	
Must establish and maintain pressure to be used as a transport mechanism	**Metric -** % change in pressure **Goal –** less than 10% change in psi over a 5 minute period
Must quickly transport material across membrane	**Metric** – transport rate (µl/min) **Goal** – 50µl to 100µl in 15-30 minutes
Must interface with a detection system capable of high sensitivity to detect proteins	**Metric** – % dilution of filtrate in buffer **Goal** – less than 1 order of magnitude dilution after filtering
Must enable high-throughput testing in order to increase our customer's current screening capacity	**Metric** - number of candidate drugs tested per week (#/week) **Goal** - 20,000 candidate drugs at a rate of 1,000 per week
Compartments (SepCons™) must accommodate a range of liquid volumes	**Metric –** volume (µl) **Goal –** 50-200 µl
Membrane must remain wet on both sides	**Metric -** % area of silicon dry during operation **Goal –** 0%

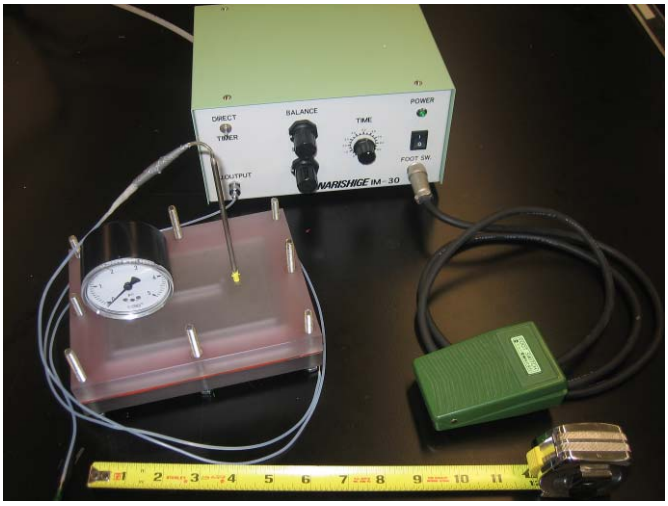
Wants	
Should be designed such that sample dilution in multi-well plate well is minimized, allowing compatibility with low-sensitivity detection systems	**Metric** – dilution in % **Goal** – maximum 30%
Limited number of components	**Metric** – number of individual components (not including SepCons™ or membranes) **Goal** – 5 components
Simple user interface	**Metric** – intuitive to veteran laboratory technician
Easy to scale up entire system to higher density plates (96-well or 384-well)	**Metric** – number of components that must be redesigned during scaling **Goal** – 0 components

Documentation of Solution

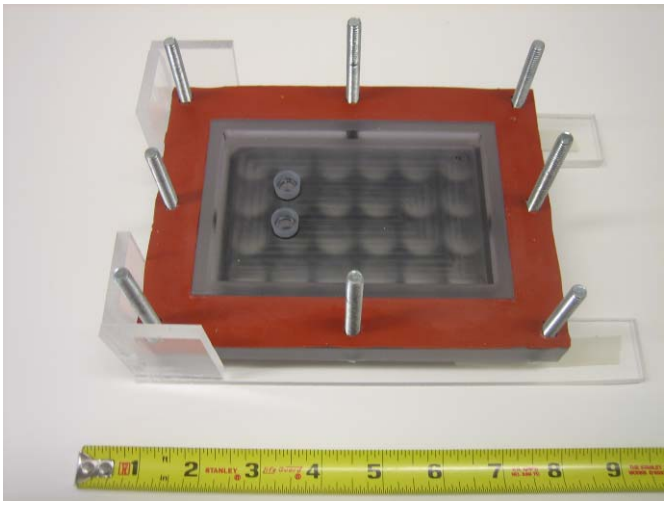
A recapitulation of our design process follows. Included are a number of photographs, design sketches, and tabulated information related to this process.



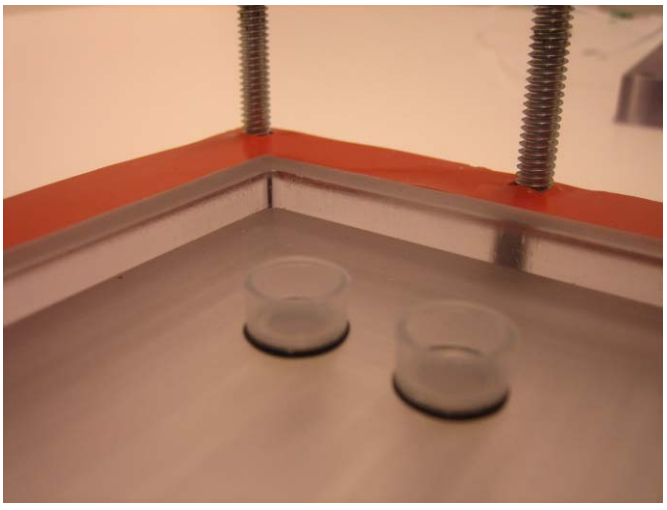
The Pressure Cap, Spacer, and Aligner are displayed in assembled form.



The assembled device is shown next to our pressure controller.



From above, the multi-well plate is seen through the Spacer; the gasket, clamping bolts, and Aligner are also visible.



SepCons™ with o-rings below them are displayed in their positions in the Spacer.

Design Constraints

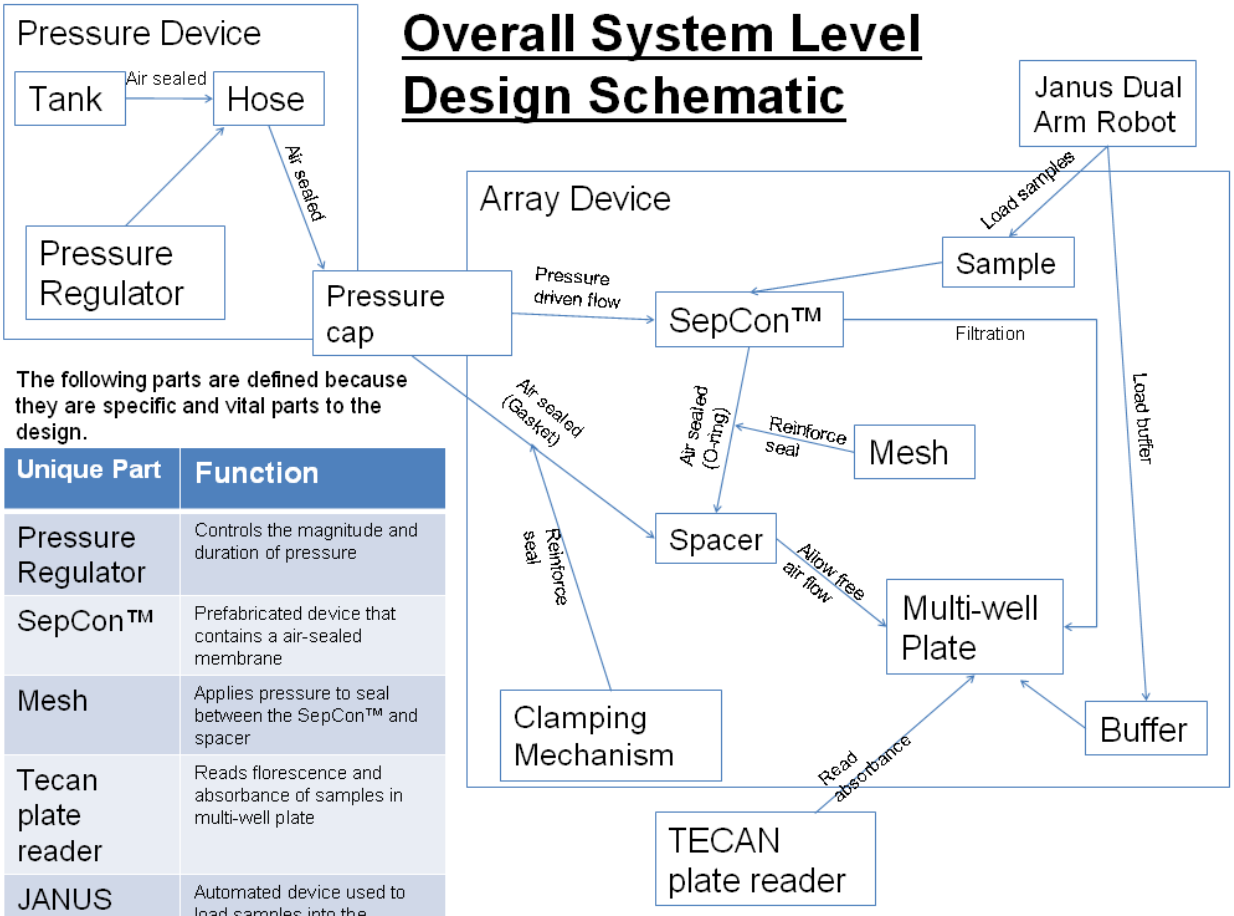
- Both sides of the pncSi membrane must remain wet at all times during testing. If prior to transport one side is not wet, capillary action will allow the pores to fill, but enormous surface

tension forces will prevent actual flow. On the other hand, if during transport one side dries out, significant drying forces are generated, causing the material to crack.[xiv]

- It is difficult, with current manufacturing, to produce a full silicon wafer in which all membranes are viable (no pinholes). Therefore, the design must account for selection of intact membranes only.
- Samples must remain physically separated from one another to avoid cross-contamination. Without this, viable test results cannot be obtained.
- The sample separation process must be completed within thirty minutes to assure minimal protein degradation.
- The device must operate normally in cold-temperature controlled environments.
- Screening capacities of approximately 1000 drug candidates per weeks should be accommodated.

System-Level Design

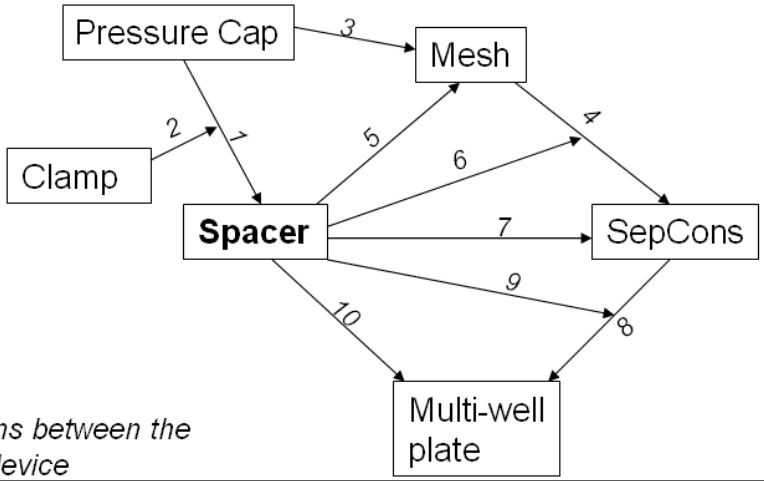
System-level design diagrams have proven highly valuable to our design process. The overall system-level schematic and magnified system-level schematic displayed below reveal all of the components of our device as well as the interactions that occur at their interfaces.



The following parts are defined because they are specific and vital parts to the design.

Unique Part	Function
Pressure Regulator	Controls the magnitude and duration of pressure
SepCon™	Prefabricated device that contains a air-sealed membrane
Mesh	Applies pressure to seal between the SepCon™ and spacer
Tecan plate reader	Reads florescence and absorbance of samples in multi-well plate
JANUS Robot	Automated device used to load samples into the SepCon™ array

Magnified System Level Design Schematic



Description of the interactions between the central components of our device

#	From	To	Interaction
1	Pressure Cap	Spacer	Pressure Cap attached to the Spacer with silicone rubber gasket in between so as to form an air-tight seal
2	Clamp	Interaction #1	Clamping mechanism (bolts, wing nuts, washers) applies pressure between the Pressure Cap and Spacer
3	Pressure Cap	Mesh	Cap applies downward pressure upon the Mesh
4	Mesh	SepCons	Mesh applies downward pressure on the SepCons
5	Spacer	Mesh	Spacer acts as a support and stop for the Mesh force on the SepCons
6	Spacer	Interaction #4	Washer and O-ring mechanism forms air-tight seal between Spacer and SepCons
7	Spacer	SepCons	Spacer holds the SepCons in the array formation
8	SepCons	Multi-well Plate	SepCons protrude into wells and deposite filtrate when running experiment
9	Spacer	Interaction #8	Spacer regulates the distance that the SepCon lies from the bottom of the plate
10	Spacer	Multi-well Plate	A user-friendly male-female fitting system ensures proper setup and allows for air flow to the atmosphere

Integration of SiMPore pncSi Nanomembranes

Choosing a method by which the SiMPore, Inc. pncSi membranes would be integrated into an array was a critical early decision. Essentially, three ideas were explored during the selection process: SepCons™, full-wafers, and multiple-nanomembrane chips. Our team ultimately selected SepCons™ as the best method for incorporating these filters into the overall design. Appendix A contains a concept scoring matrix and other data that factored into this decision.

SepCons™

Detailed drawings of SepCons™ can be found in Appendix B. They are comprised of a plastic cylinder open to the atmosphere on one end and closed off by a nanomembrane on the other as shown in Figure 2 below.

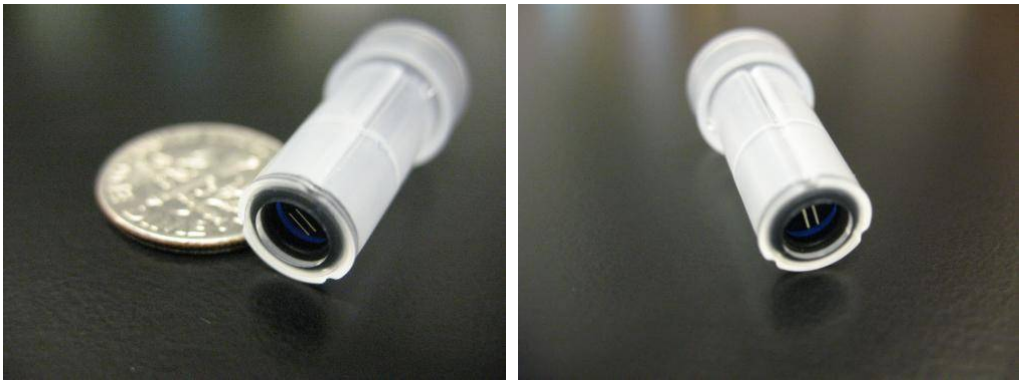


Figure 2[xv]: Images of SepCons™ with SiMPore, Inc. nanomembranes in place.

Fluids are inserted into the empty plastic cylinder and will cross the membrane by any number of transport mechanisms, which will be discussed in greater detail later. Due to their somewhat bulky nature, our team was not willing to commit immediately to the use of SepCons™. Several specific drawbacks attributed to the use of SepCons™ were discussed at length by the team members.

First, at a cost of several dollars per SepCon™, we were skeptical that implementation of this design would allow us to stay within our budget constraints. However, the realization that SepCon™ donations from Dr. McGrath would allow for prototyping and testing of our device made this less of a concern. Next, concerns were expressed regarding the time requirement for SepCon™ assembly – the individual parts can be ordered from a manufacturer, but the user would be required to insert the desired membrane and put together the final product. Appendix C contains several pictures documenting the assembly process. As a high-throughput design, one of our major goals is to produce a device that would minimize the run-time for drug screening. The use of SepCons™ seems to be at odds with this objective. Finally, as a "company" trying to market a state-of-the-art product and earn profits, our team members felt that SepCons™ represented the least elegant solution to the problem at hand. Although this concern does not affect the function of our design, it seems valid to consider the attractiveness and marketability of our design components.

In contrast to the disadvantages that were considered, several characteristics of SepCons™ were highly favorable to our design. Perhaps most importantly, SepCons™ allow for individual selection of intact nanomembranes. This is necessary since they tend to express a high rate of failure as a result of the manufacturing process. Thus, SepCons™ promote high-throughput drug screening by guaranteeing that all array locations can be used, and also the establishment of a pressure gradient (broken membranes will not support differential pressure). Also, SepCons™ are highly effective at preventing sample cross-contamination due to the position of the samples at the bottom of an approximately 1 inch deep well. Sample quality is of the utmost importance when testing drug efficacy using highly sensitive assays. Furthermore, the elongated cylinder shape allows for the insertion of the sample into a filtrate collection area (multi-well plate), and gives us increased control over filtrate dilution by allowing us to vary the volume of buffer added to the collection wells. On the same note, the shape also allows for easy establishment of a pressure gradient. By leaving space in between the SepCon™'s walls and the

multi-well plate, the filtrate collection zone can be exposed to atmosphere; and, the tightly sealed membrane at the bottom of the SepCon™ favors pressurization of the SepCon™ and its contents.

Full-Wafer of pncSi Membranes

As an alternative to the SepCon, our group proposed the use of a full-wafer design. In this design (Figure 3) a flat, single unit with numerous nanomembranes patterned into its surface would constitute the array to be used for filtration.

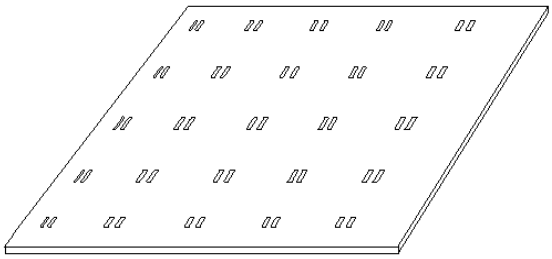


Figure 3: Sketch of full-wafer of pncSi membranes.

Our team considered this method of integrating the nanomembranes into our device for several reasons. This design would eliminate the assembly time requirement introduced by SepCons™. Also, it would allow us to avoid purchasing the plastic SepCon™ components, which would mean decreased manufacturing expenses. Finally, the fact that all of the membranes would be contained within a single, continuous element would make it easier to implement a pressure-driven flow transport mechanism by eliminating the need for pressure tight seals between multiple membrane array constituents. Yet, in our meetings we identified a number of drawbacks to this design. This concept would require our team to custom design and order wafers from SiMPore, Inc. rather than using individual stock wafers as with the SepCon™ design, resulting in increased cost for the membranes. Another notable problem with this design option deals with the low reliability of membrane manufacturing. This would make it more difficult to achieve our goals of high-throughput screening (we would have to inspect every membrane and a significant proportion would be unusable) and using a pressure gradient to drive fluid flow (again, the broken membranes would not support differential pressure). Also, the lack of a physical barrier between samples – which would be placed as small-volume droplets on top of each functional membrane – is another problem with this design. The risk of cross-contamination would be greatly elevated over the SepCon™ design concept. As a solution, one can imagine placing some sort of hydrophobic webbing atop the wafer to reduce this risk; however the result would still not be comparable to that provided by SepCons™. Finally, as with the SepCon™ design, the shape of the full-wafer design needed to be assessed. Since the wafer is completely flat both on the top and bottom (aside from the small membrane indentations), this concept would not allow for insertion of the samples into separate wells for filtrate collection. Thus, it would be difficult to keep the backside of the membranes wet, and it is hard to imagine a feasible system for collecting the filtrate. The inability to insert our membranes and samples into the filtrate collection zones would also greatly decrease our control over filtrate dilution.

Multiple-Nanomembrane Chips

Figure 4 shows a simple sketch of the third candidate for solving our membrane implementation problem: multiple-nanomembrane chips.

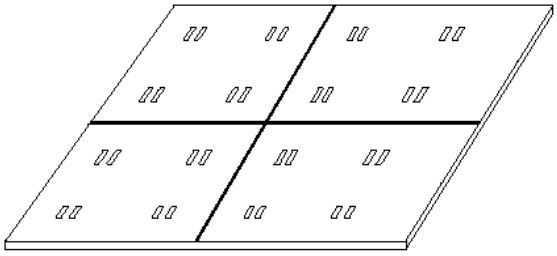


Figure 4: Sketch of multiple-nanomembrane chip design.

Much like the full-wafer design, the multiple-nanomembrane chip design utilizes a number of flat sections of wafer with several pncSi membranes patterned into each chip. This concept expresses almost exactly the same advantages and disadvantages as the full-wafer design, with a few exceptions. By using smaller chips arranged adjacent to one another, we could achieve the same layout as with the full-wafer concept, while at the same time eliminating the possibility of having broken membranes incorporated into the array. To avoid being stuck with broken membranes, we would divide the full-wafers into several chips and select only those chips with all intact membranes. These selected chips would then be geometrically combined to create the array.

Unfortunately, along with this improvement came two large problems. First, the cost for purchasing the nanomembranes from SiMPore, Inc. would most likely surpass that of the full-wafer design due to the membrane selection process. Like the full-wafer concept, the multiple-nanomembrane chip solution would require custom designed membranes to be ordered rather than stock parts; on top of this, our selection process for eliminating broken membranes would necessitate the use of more wafers than the full-wafer design because many chips would be discarded. Another problem with this concept relates to the application of pressure-driven flow. Since the chips are not one continuous component, we would require an air-tight seal between each chip to allow for differential pressure to be applied. As displayed by Figure 4, creating a method for reliably sealing these chips would be difficult and expensive.

Loading

One of the defining characteristics of a high-throughput screening system is the use of robotic liquid handling devices.[xvi] Thus, in keeping with one of our overarching goals of creating a high-throughput system, it has been important to consider the manner in which samples of any kind will be loaded into the Nanomembrane Array for each run. Typically, samples are loaded into multi-well plates using a hand-pipetter and proper liquid handling techniques. This practice is acceptable for tests with low numbers of trials, but certainly does not lend itself to high-throughput testing of thousands of candidate drugs. As a result, we considered other methods for high capacity laboratory automation.

Our customer, Dr. Harold Smith, has recently added an automation system to his laboratory. This device, the JANUS Automated Workstation, is capable of accurately and efficiently handling small volumes of liquids and interfacing with common labware (i.e. multi-well plates, pipette tip dispensers, etc.). The choice to integrate this device into our design was a simple one. The increased speed, accuracy, precision, and repeatability that this laboratory automation device affords are undeniable, and especially useful in a high-throughput system application.

We acknowledge the fact that while our customer is fortunate enough to have this system in his lab, other users may not. For this reason, have ensured that hand-pipetting is also compatible with our device. Multi-well hand-pipetters and other brands of robot dispensers are compatible with our device since the spacing of wells on 96-, 384-, and 1536-well plates is nearly standard across all manufacturers, and so too is the spacing of the pipette tips on lab automation systems.

Since the JANUS system is intended for use in true high-throughput applications, it is not sold with dispensers for 24-well plates. Rather, it accommodates 96- and 384-well formats. To overcome this obstacle for prototyping purposes, we have mapped out the well locations for 96-well plates and placed the SepCon™ holes in our array such that they will both fit into a 24-well plate and accept pipetting from 96-well pipetters. Although this does not at all represent an ideal solution, we expect that future models of our device will not face this dilemma as a result of incorporating 96-well plates into the design.

In terms of actual interactions with the JANUS Automated Workstation, our Nanomembrane Array device only requires two loading steps. First, the multi-well plates will be placed on the JANUS' loading table and filled with the correct volume of buffer. Also, the Spacer component with SepCons™ already inserted into the holes of the array will be placed on the JANUS' loading table and the SepCons™ will be filled with the appropriate amount of sample. Note that in order to prevent the bottoms of the SepCons™ from touching the loading table we have created small legs on the corners of the Spacer component. These will afford us sufficient clearance. Of course, Dr. Smith, as well as any other customers using similar laboratory automation devices, will be using the JANUS for several other portions of his screening process – dilution, sample preparation, addition of drug candidate to sample. However, since these steps would have to be completed regardless of how our device operates they were not of great concern to our design process. Following the loading of the multi-well plate with buffer and the Nanomembrane Array with sample, the user would remove these two components from the JANUS and assemble the device.

Transport

In order to ensure that drug screening is conducted by our device quickly enough to prevent denaturing of proteins and without introducing factors such as temperature, pH, or other changes that could impact the results, the choice of an appropriate method for transporting candidate drug, protein, and buffer across the membrane was critical. Our design team ultimately decided to use differential pressure in this application, however several other options were investigated. Appendix D contains a concept scoring matrix that was used in this selection process.

Diffusion

The use of diffusion as a means of sample transport was the simplest option. Although it may seem that diffusion should have been crossed off the list immediately, it actually brings at least one crucial advantage to the table. Because of the relative frailty of the pncSi membranes that our device uses to separate molecules in the sample, diffusion guarantees that none of these membranes will burst during a test. While this characteristic of diffusion is extremely important to the validity of testing done with our device, numerous drawbacks steered us away from diffusion. Low sample flow rates, include an increased risk of sample degradation, and a decreased ability to make our device high-throughput were factors that prompted us to explore other options.

Electrophoresis

Biochemists and other scientist dealing with proteins utilize electrophoresis frequently (for example in SDS-PAGE experiments). Its principals are not too complex: apply an electric field throughout a solution containing charged particles and these particles will migrate via repulsive and attractive forces towards either the positive or negative terminal. We considered using electrophoresis in our device because it is so widely used and has been proven effective in certain applications. However, upon further consideration we identified a few drawbacks.

Electrophoresis requires knowledge of the charge of the particle of interest in order to achieve flow in the proper direction. Gaining this knowledge not only complicates the use of our device, but also makes our device less attractive to potential customers. Electrophoresis would require individual users to characterize the particles for their application, potentially change the orientation of the electric field depending on this charge, alter the electric field strength, and if the particle of interest had either a neutral or negligibly small charge electrophoresis might not work at all. As with all of our design selections, we sought to make a transport method decision that would not limit our potential market. In order to stick with this mindset, we were unable to select electrophoresis because it could preclude many customers.

Electroosmosis

Electroosmosis employs electrically induced bulk fluid flow through the silicon pores of our membranes to achieve molecular separation. Unlike electrophoresis, which induces bidirectional motility (depending on charge) in specific ionic species, electroosmosis achieves predictable, unidirectional bulk flow as a result of silicon's unique properties. Figure 5 diagrams the mechanism and flow characteristics of electroosmotic flow in pores.

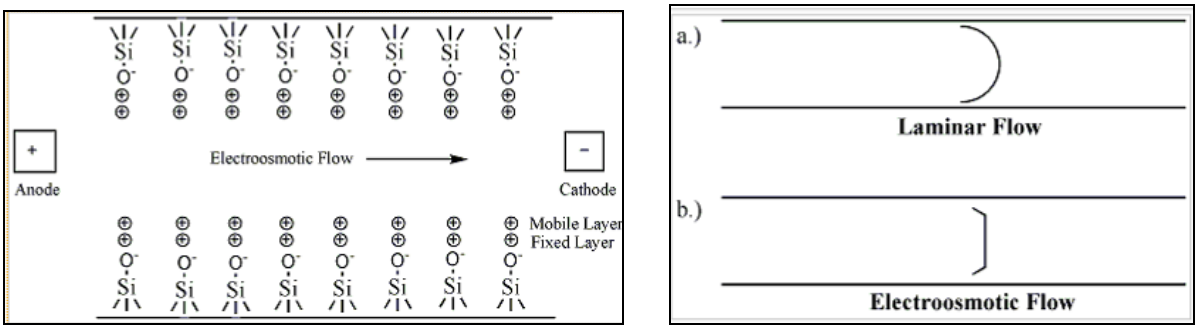


Figure 5[xvii]: On the right, the basic principles of electroosmosis are displayed, including the establishment of sianol (Si-O^-) groups along the pore walls which attract cationic fixed and mobile layers. On the left, electroosmotic flow profiles are compared to laminar, pressure induced flow profiles.

As displayed by the figure above, electroosmotic flow truly utilizes the pncSi membranes to their full potential by the creation of sianol groups that allow for bulk flow. In addition, electroosmosis allows for very high flow rates compared to all other transport options while at the same time providing low risk of the membranes bursting. Unfortunately, due to the fact that the SiMPore, Inc's pncSi membranes are fairly new, electroosmosis has not been studied extensively in conjunction with them. As a result, solutions to electroosmosis' technical problems – such as corrosion of electrodes in solution (except when the expensive metals Au and Pt are used), pH and temperature extremes in the vicinity of the electrodes, and user safety – have not yet come about.[xviii] Thus, electroosmosis was the most elegant sample transport option. But, because of many technical problems associated with electroosmosis, we chose to set it aside in the prototyping phase, with the hopes that future versions of our device will utilize this promising technology.

Differential Pressure

The use of a pressure gradient for fluid transport is not at all uncommon. In fact, it is utilized in nearly every fluid flow situation that we encounter regularly. However, two major obstacles stood in the way of implementing pressure-driven flow in our device. First, creating and maintaining an elevated pressure was expected to be a difficult challenge. Also, keeping this elevated pressure high enough to substantially increase flow rates, but at the same time low enough to avoid bursting the membranes was an absolute requirement. The producer of these membranes, SiMPore, Inc., indicates that the burst pressure is approximately 1atm/0.2mm^2.[xix] We will be using 0.4mm^2 membranes at a pressure between 0 and 3 psi. This leaves us well below the burst pressure. Also, the SiMPore, Inc. website lists H_2O permeability in terms of differential pressure to be 10mL/(ATM*cm^2*min).[xx] At the 0.4mm^2 membrane area and 3 psi pressure level that we will be using, this corresponds to 8.5µL/min. Fortunately, previous investigation by members of Dr. James McGrath's (our advisor) laboratory has yielded positive results regarding pressure-driven flow, and revealed the feasibility of this transport mechanism. As a result, we chose to pursue differential pressure as a means of sample transport for our prototype device.

After concluding that differential pressure would be used as a transport mechanism, our design group had originally planned to use N_2 gas supplied by a large compressed gas cylinder. Dr. James McGrath, our supervisor, had offered to loan most of the necessary pressure system components

associated with this N_2 supply, including nozzles, regulators, and some tubing. Unfortunately, due to the space constraints of the senior design laboratory and the logistics of moving such a large pressure system, we were unable to make use of this pressure source. As an alternative, our design team decided that for prototyping purposes the standard laboratory compressed air supply would suffice in conjunction with a pressure controlling device supplied by Dr. McGrath. This microinjection pressure controller, made by the Narishige Group, was connected through tubing to the compressed air supply and then to our Nanomembrane Array via smaller diameter tubing and a threaded piece. It is unique in that it allows the user to supply air at either constant flow or, using a foot pedal, as a burst. The design team felt that these control options would be ideal for quickly pressurizing our device and then maintaining pressurization for an extended period of time.

Our Device Components

Key Elements of Each Component/Important Design Decisions

Refer to Appendix E for individual component mechanical drawings.

Pressure Cap

The "Pressure Cap" we constructed for our device had several key constraints that were crucial to consider during its development. Obviously, the Pressure Cap would need to capable of establishing, maintaining, and controlling a pressure differential. In order to reach these goals, the Pressure Cap would need to interact with the other pieces of the device, as well as the chosen pressure system.

The Pressure Cap was made of polycarbonate, chosen largely because it is work-hardened and is less likely to crack during machining. A cavity was removed from one side of the polycarbonate to allow a chamber for the pressure differential to be contained within. The size of this cavity was not particularly crucial, but it needed to be large enough to allow the pressure differential to be applied to each membrane within the array. Figure 6 displays the working prototype Pressure Cap with a low pressure gauge and air supply connection attached.

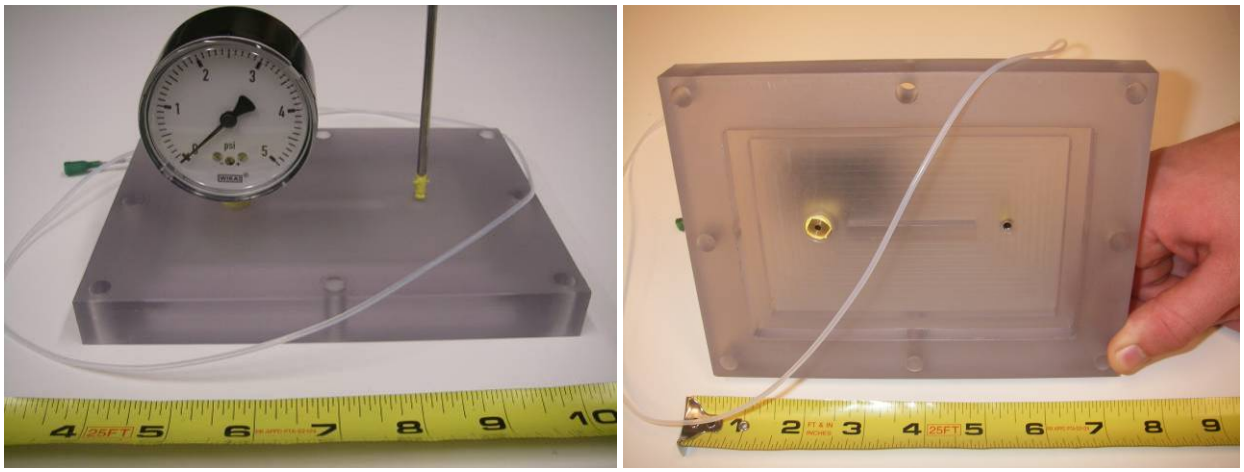


Figure 6: Shown above is the Pressure Cap component.

Establishing the pressure differential for our device would require interaction with a pressure source, such as compressed air, nitrogen, helium, etc. For our experiments, we decided to use the compressed air flow available in the senior design lab. Incorporated into the Narishige pressure controller that would be used as our pressure regulator is a pressure injection piece, threaded on one end. A hole was tapped into the top of the Pressure Cap that would allow this injection piece to be screwed into the cap, and, using Teflon tap on the threads, the pressure could be sealed within the cap.

The Pressure Cap also interacts with the Spacer which aligns below the cap. A ledge of equal size on the outside of both the Spacer and the cap allows a region for a gasket material to be placed as well as a clamping mechanism. This ledge is crucial to develop the pressure seal around the edge of the Pressure Cap. A number of clamping mechanisms were envisioned to be compatible with this ledge, including using actual clamps, bolts, or other methods. For the prototype, holes were drilled through this ledge that would allow bolts secured into the Spacer to pass through. Once these bolts are tightened using wing-nuts, the pressure needed to generate the seals would then be applied.

The mesh piece, which would allow another pressure seal to be created between SepCons™ and Spacer also interfaces with the Pressure Cap. The cap applies the pressure to the mesh necessary to generate the seal. A groove was cut into the cavity of the Pressure Cap, which allows downward force to be applied to the outside edge of the mesh when clamping occurs. In an effort to minimize bowing in the Spacer, a rectangular bar was left in the top of the cavity that also allows downward force along the center of the mesh.

The proposed pressure to be applied within the Pressure Cap is 1 - 3 psi, and in order to carefully monitor the pressure differential a pressure gauge was incorporated into the cap. In a similar fashion to the injection instrument of the pressure system, a hole was tapped that would allow the pressure gauge to be screwed into the cap with Teflon tape to establish a seal and prevent the loss of pressure. This gauge is an important component in monitoring the pressure differential and testing the ability of the device to establish and maintain pressure.

Careful detail was put into the dimensions that would govern any interaction with a sealing mechanism. Other dimensions were less important, and were largely chosen to improve interaction with the various elements of the device or to reduce machining costs.

Spacer

The “Spacer” is arguably the most important component of our device, as it is responsible for physically holding all of the SepCons™ and providing a means for integrating the pncSi membranes into the array. For this reason, the Spacer required the greatest share of our design effort, undergoing several iterations before arriving at its current form. We considered two other options for integrating membranes into the array before deciding to use SepCons™ – full- wafers, or multiple-membrane chips. These alternative designs were discussed previously. As a reminder, all of our concepts were evaluated on their ability to enable both sides of the membrane to remain wet, keep samples physically separated to avoid cross-contamination, decrease sample dilution, and to accommodate transport of material using a differential pressure system.

As mentioned previously, both of these alternative design concepts were ultimately eliminated in favor of the SepCon™ concept. SepCons™ provide a solution that is able to physically separate samples (to eliminate cross-contamination), allow both sides of the membrane to remain wet, and can accommodate variability in wafer manufacturing. A rendering of the final Spacer design can be seen in Figure 7.

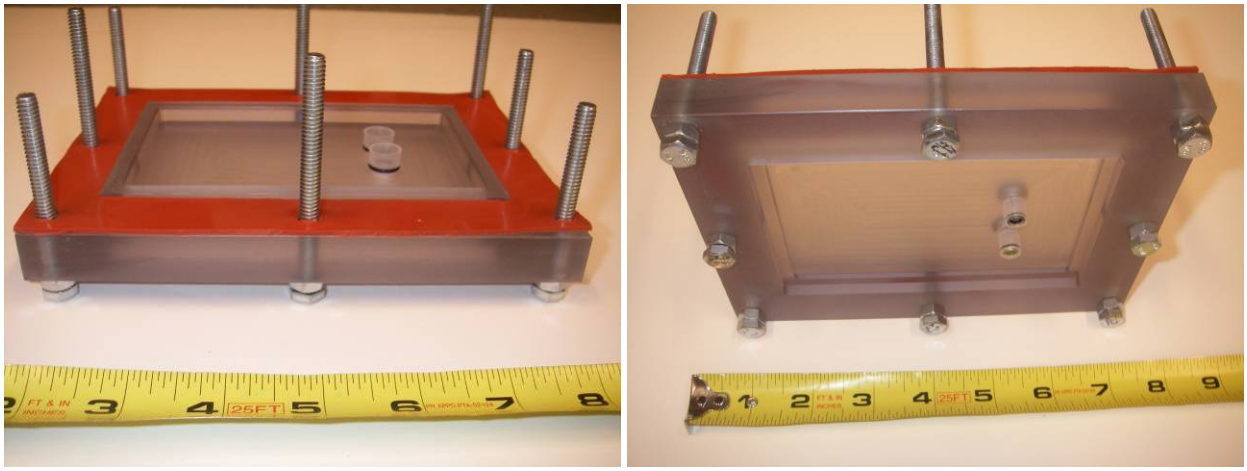


Figure 7: The prototype Spacer component.

The Spacer interfaces with both the Pressure Cap and the multi-well plate, so it was designed such that each component fits together properly and is correctly placed by the user. The top surface has a ledge for the Pressure Cap, and the bottom surface has a recess to accept the multi-well plate. The inside of the Spacer is intended to have 24 holes drilled through it that correspond to the 24 wells of the multi-well plate. Each SepCon™ contains a single intact membrane and is placed in a hole in the Spacer, extending down below the Spacer into the wells of the multi-well plate. Since the SepCon™ extends into the well below it, buffer solution in the well keeps the back side of the membrane wet during operation. Figure 7 shows that our prototype device only has two SepCon™ holes in place. We decided not to go forward with drilling the rest of the 24 SepCon™ holes due to concerns over the introduction of pressure leaks. However, our pressure testing revealed that the presence of these holes did not detract from our device's ability to remain pressurized whatsoever. Therefore, in the immediate future we will most likely complete the Spacer component by adding in the rest of these holes.

An important area of design selection involved deciding upon a sealing mechanism that will maintain the pressure differential between the Spacer and individual SepCons™. Initially, it was discussed that the entire Spacer could be covered with a sheet gasket material or PDMS and holes for the SepCons™ could be cut into the material. In order to achieve an airtight seal, compression would then be applied to the cap and the mesh integrated into the cap, which would compress the SepCons™ into the gasket. However, forming such a gasket to our device was expected to be difficult and there would be little control over the thickness of the gasket, especially when using PDMS as it must be cured and leveled while being heated in an oven. This would provide lesser control over the depth of the SepCons™ in the wells of the multi-well plate. Furthermore, we were concerned that compressing the

sheet gasket would deform the punched holes in a non-uniform way. Although this would occur at very small scales, we decided that we should still avoid the potential leaks. This phenomenon can be seen in Figure 8, but has been exaggerated for clarity.

Besides the use of a sheet gasket, the use of o-rings was discussed. This seemed like a better solution as each SepCon™ would be treated individually with a tight seal formed by the o-ring. The size of the o-ring that we choose has an inner diameter such that, once placed on the SepCon™, the o-ting stretches by about 5% (close to 2%, the recommended stretch).[xxi] The material for the chosen O-Rings is acrylonitrile-butadiene rubber (NBR), a common o-ring material chosen for its material properties, largely the ability to withstand cold temperatures (-22°F) and its high compression set resistance, tear strength, and abrasion resistance.[xxii] Some common standard elastomer tests that are used in characterizing the o-rings include a test for hardness (ASTM D 2240), tensile strength (ASTM D 412), temperature retraction (ASTM D 1329), compression set (ASTM D 395), and tear strength (ASTM D 624).[xxiii]

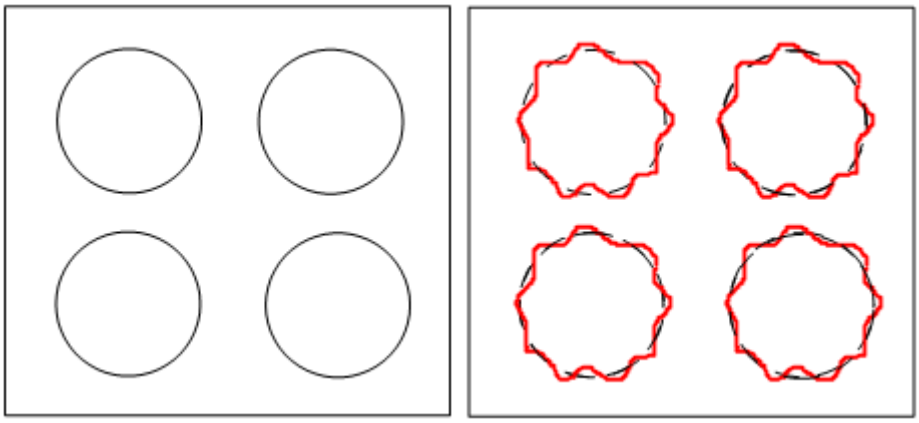


Figure 8: PDMS gasket at rest (left) and with pressure applied (right).

Mesh

The "Mesh" is a vital component to ensure pressure seals are formed around the base of each SepCon™ with the Spacer. The purpose of the Mesh is to apply an even downward force to each SepCon™, compressing its respective o-ring. It was designed to fit tightly inside of the Pressure Cap, resting on a small ledge and also supported by another bar in its center to prevent bowing. We chose to manufacture this component out of acrylic due to its relatively high stiffness, low cost, and transparency. The Mesh should never come in contact with samples, so it will not usually need to be sterilized. For this prototype, the Mesh and Pressure Cap are two separate components, due mainly to ease of production, but in a future version they will be integrated into a single piece. Refer to Figure 9 below for a photograph of this component.

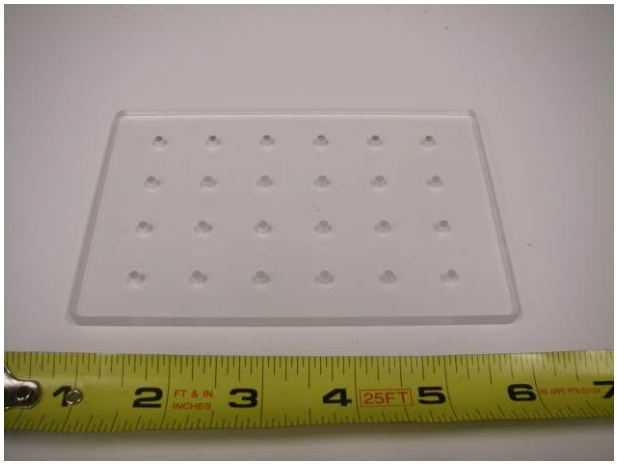


Figure 9: Nanomembrane Array's Mesh component.

Aligner

The "Aligner" is a piece that we decided to construct after demonstrating our device to our customer, Dr. Harold Smith, partway through the semester. At the time of the demonstration, we were using only mock-up components since our actual parts had not returned from the machine shop. Many times when we tried to place the Spacer with SepCons™ on top of the multi-well plate, we bumped the bottom of a SepCon™ on the rim of a well. Quickly, we realized that this physical contact could dislodge a SepCon™ from the Spacer, spilling its contents, and causing risk for cross-contamination.

As a solution, we concieved of a simple piece that would help the user perfectly place the Spacer on top of the multi-well plate. The Aligner is a rectangular piece of acrylic with an area removed to accept the multi-well plate and two 90^{o} corners to guide the placement of the Spacer. The user slides the multi-well plate into place, pushes the Spacer flush against the corner guides, then lowers the Spacer carefully into place. Double-sided tape is currently used to provide slight resistance to sliding around on the workspace. The Aligner can be seen in Figure 10.

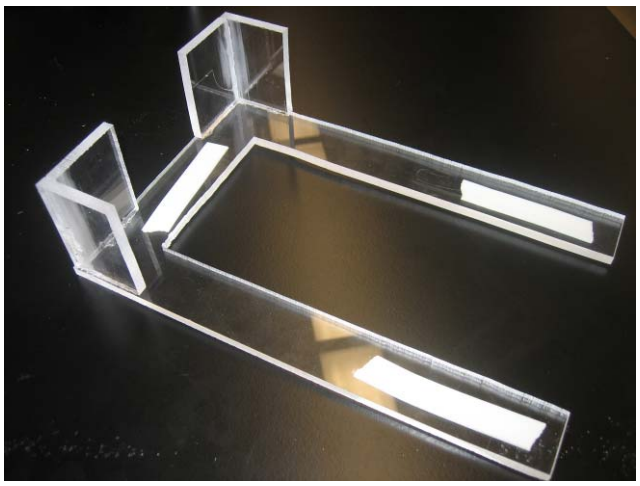


Figure 10: The Aligner component is shown above.

Filtrate Collection

The choice of a filtrate collection mechanism was also important to our design process. For our customer, Dr. Harold Smith, this filtrate will be LMM APOBEC3G protein. However, our aim, as with the entirety of our device, was to select a design that could be used for a range of applications. Our team weighed the use of multi-well plates against the use of custom mechanisms. In the end, multi-well plates were selected.

Multi-well Plates

Multi-well plates have a lot of advantages for our application. They are reliably manufactured, robust, cheap, require no assembly, and they come in a multitude of shapes, sizes, and colors. Also, very importantly, they are almost guaranteed to be compatible with the other devices with which our array will interface.

However, certain disadvantages are introduced by this filtrate collection mechanism as well. Most importantly, their uniform layouts provide us with little room for customization of our membrane array, and it was difficult to imagine a method for interfacing multi-well plates with any membrane integration mechanism other than SepCons™. Of course, the decision to use SepCons™ eliminated this latter concern (and, in fact, favored multi-well plates). Also, because of the wide range of multi-well plates that are available, deciding upon an exact multi-well plate type for use posed another dilemma. As a high-throughput device, we initially envisioned using 96-well plates for our array. But, a simple examination of the dimensions for both the wells and the SepCons™ rendered this option impractical. The SepCons™, as currently manufactured by SiMPore, Inc., are too large to allow for a 96-well plate to be used. Therefore, our team switched our focus to 24-well plates. These have larger diameter wells and more spacing between the wells meaning an easier fit for the somewhat bulky SepCons™. Unfortunately, this detracts from our device's ability to be high-throughput. However, our team was content with making this sacrifice for the prototype stage. We imagine that more refined, future versions of our product would be designed to incorporate greater drug-screening capacity per trial (i.e. 96- or 384-well plates), maybe incorporating a SepCon™-like device that can be more easily interfaced with small wells. In addition to selecting the number of wells each plate should have, our team has decided to use multi-well plates that have black walls and clear bottoms. These characteristics were important for our design because the use of fluorescence detection mechanisms would have potentially led to cross-talk if clear-walled plates were used. Thus, our final filtrate collection choice was a 24-well, black-wall, clear-bottom plate. Appendix F contains dimensioned drawings for the particular plate used in out prototype. Most similar 24-well plates have nearly identical dimensions.

Novel Alternative Design

Prior to finalizing our selection of multi-well plates, our team brainstormed an alternative collection mechanism. This alternative was conceived almost entirely to cater to the full-wafer of nanomembranes and multiple-nanomembrane chip designs, since the interface between the filtrate collection mechanism and the membrane integration mechanism was of such concern. In essence, we proposed the use of rubber webbing that would separate each pncSi membrane and was to be glued or clamped in place below the membrane wafers. This rubber webbing would then be sandwiched in place

by clamping a glass piece below both it and the membranes in order to create individual, water tight compartments for filtrate collection. Additionally, we envisioned the glass component allowing for easy detection following the filtration step. As one can imagine, it quickly became apparent that this design presented many flaws. Although it provided us with a solution to the interface between the flat-bottomed wafers and the filtrate collection area, this concept introduced immense complications to the setup procedure, cost of production, use of pressure-driven flow (the collection zones would not be open to atmosphere), and other areas of our design. Because of this vast number of issues, our team decided that multi-well plates were a superior solution.

Detection

Although our device works independently of its associated detection system, the marketability of our device relies upon compatibility with a mechanism of detection. Key constraints taken into account when selecting detection techniques are sensitivity, ease of quantifying results, the speed and simplicity of detection mechanisms, and the reproducibility of results. Aiding our group in detection, the Tecan Microplate Reader is a state of the art absorbance and fluorescence detector, provided by Dr. James McGrath's laboratory. This machine is functional with most standard assay techniques and multi-well plates, allowing for accurate measurements for our testing. Many possible detection techniques were considered. These include FRET, ELISA, general protein assay, Quant IT imaging, and Bradford assay techniques. As a group, we concluded that the optimal techniques for our detection system would be a Bradford assay or Quant IT.

FRET (Fluorescence Resonance Energy Transfer)/ELISA (Enzyme Linked Immunosorbent Assay)

In our initial search for a detection system, we looked to modern methods of biochemical protein assays for guidance, specifically FRET and ELISA imaging. Both of these systems require fluorescent antibody development specific to the drug to be tested, a large supply of expensive reagents, and consultation with individuals of greater expertise in biochemistry. Although these tests would display the most robust and accurate results that we could be looking for, in the grander view of HTS, such accuracy is not required theoretically used solely for a binary response to drug candidate efficacy (required for primary screening). Furthermore, the development of the reagents needed for such assays consume valuable time and resources that could be spent more wisely. Additionally, the production of these antibodies sometimes result in a lack of completely specific binding, leading to possible false negatives, which are much more hazardous than false positives in our results. Figure 11 below displays the basics of both FRET and ELISA.

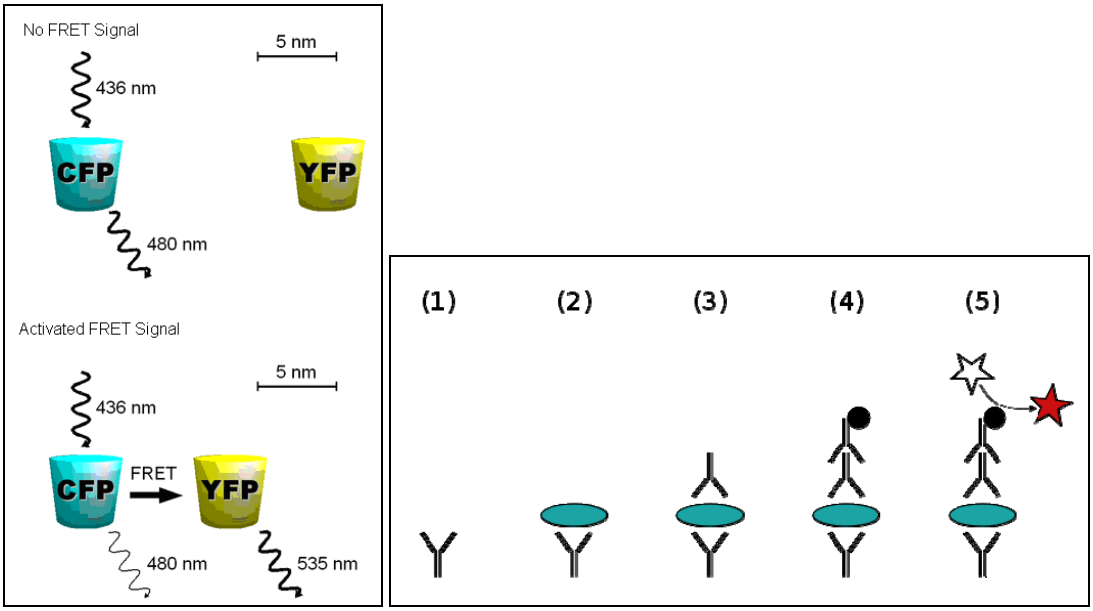


Figure 11: Left, FRET imaging consists of the binding of the CFP associated molecule to the necessary protein then the subsequent interaction with the YFP molecule to release a different wavelength of light. Right, ELISA sandwich staining (1) plate is coated with an antibody; (2) antigen or protein binds to the antibody; (3) an antibody specific to the antigen binds to the upper side; (4) a secondary antibody with a fluorescent subgroup binds to the previous antibody; (5) certain wavelength light is given off from the sample.

Finally, these methods of protein assay do not make use of the advantages that our system delivers to the field of biochemistry. The effort of incorporating a pncSi membrane into our system, is derived from the specificity of that component. With this specificity obtained by separating molecules by molecular size as opposed to epitope reactivity, we have the ability to bypass labeling methods currently used. This in turn cuts the cost of running tests greatly, as well as adding a new way of testing. Detection methods such as FRET and ELISA are effective with our process if one is attempting to differentiate between two similarly sized molecules. However for differently sized molecules that can be sorted via our system, a more general protein assay would suffice for a HTS system.

General Protein Assays/Quant IT Imaging/Bradford Assay

As an alternative to the more specific assay techniques, general protein assays, such as the general protein assay (GPA), Quant IT staining and Bradford assays, prove to be easier and more cost effective than tests with more specificity. In these assays, there is a dye that undergoes nonspecific binding amine-containing molecules, as opposed to the antibody binding in FRET and ELISA. This does not show how much of a specific protein there is in the solution, but tells what the total protein concentration is in the solution. This reading however is sufficient for our high-throughput filter device, very cost effective, and less cumbersome for the lab technician.[xxiv]

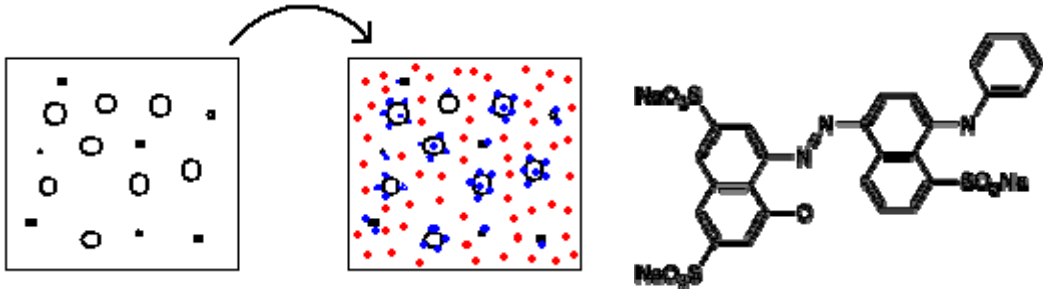


Figure 12: At left, it is seen that upon addition of Bradford reagent (Coomassie Blue) to the sample, bound particles transmit blue colors (595 nm) while unbound particles transmit red colors (465 nm). At right, the Bradford Assay reagent coomassie blue is displayed.

There are a few problems that arise when working with a general protein assay. Since the only specificity we would be utilizing is that of the membrane pore size, smaller unwanted protein particles can pass through leading to possible false positive results. Also, as in all biochemistry experiments, there runs the risk of contamination due to equipment or human error, introducing foreign proteins that could also lead to false positives.[xxv] These factors, however, are not projected to decrease the efficacy of our results in a HTS system. We have attempted to make our device as user-friendly as possible, and to automate the system with a JANUS Automated Workstation and Tecan Microplate Reader to avoid possible risks of contamination.

There are distinct differences between the GPA, Quant IT and the Bradford assay. GPA and Quant IT both work based on fluorescence readings, while Bradford Assays use absorbance to measure concentration.

As stated before, the Tecan Microplate Reader can make accurate measurements of the absorbance or the fluorescence of a sample. However, there a fundamental differences between these two methods of imaging. Fluorescence imaging can take readings of concentrations up to 3 orders of magnitude less than those of absorbance[xxvi]. This is important when working with very small concentrations of samples.

However, fluorescence imaging requires much more labor intensive protocol due to the fact that it cannot come in contact with ambient light. Exposure of fluorescent compounds to light causes photobleaching and loss of effectiveness of the molecule. This therefore requires always keeping the reagent in a dark, cool room or refrigerator, in a non-transparent container. Furthermore, when testing, the lab technician must either work in a dark room, or continuously cover the samples. Also, absorbance dyes are more cost efficient than fluorescent dyes due to their reactivity with light.

Our team has decided to undergo testing with Bradford Assays, because we have calculated that the concentration of samples in our device will be large enough, at least in initial testing, to avoid the cost of fluorescence staining molecules. Furthermore, with our limited experience with biochemical assays, the protocol for the Bradford Assay is by far the simplest of any assay. Our current data for the Bradford Assay using Bovine Serum Albumin (BSA) reinforces that sentiment.

For our customer, Dr. Harold Smith, we will suggest that he use a fluorescence reading system for his results, such as the Quant IT. The cost of his proteins, hAPOBEC3G, outweighs the cost of the detecting reagent, while in our case, BSA and Cytochrome-C are very inexpensive. By using an imaging technique that is orders of magnitude stronger, he can use much less concentrated samples of his protein loaded into the SepCon™. Furthermore, it is not fully known how higher concentrations of protein will inhibit flow through a membrane, but more testing is needed to determine the severity of this constraint.

A unique characteristic of the detection system of our device is that it will merely be a suggestion to the customer on how to use our product. Our assay technique can change in the future very easily without hindering the efficacy of our device.

Nanomembrane Array Protocol

The following represents a generic protocol for the operation of our prototype device. This protocol does not address the use of either the JANUS Automated Workstation of the Tecan Microplate Reader; instructions and user manuals for these two peripheral devices accompany them in their respective laboratories (see supervisors Dr. Harold Smith and Dr. James McGrath).

Calibrating the Pressure System

1. Insert SepCons^{TM} containing blank silicon chips (silicon wafers with no membranes) into Spacer and assemble device components (Pressure Cap, Mesh, Spacer), tightening screws to establish the seals.

2. Connect Narishige pressure device to the Nanomembrane Array via the pressure injector connected to the pressure cap, ensuring that the initial flow is set to 0.

3. Slowly apply pressure using balance until the desired pressure is reached and maintained within the cap. The pressure should fall within the range of ~1-2psi.

4. Secure the balance knob in the locked position to preserve the desired level of pressure.

5. Remove Nanomembrane Array from the Narishige pressure device.

Preparation of Protein Samples/Buffers

1. The preparation of protein samples is dependent on the specifications for the protein being used and the detection reagents to be utilized. One example is the use of Cytochrome-C, which is diluted from a stock solution of 10mg/mL to a concentration of 16ug/mL before being filtered in order to give a readable concentration for a Bradford Assay. The dilution of the filtrate and the linear range of the detection reagents must be taken into account when determining the concentration of protein to be loaded into the device.

2. The buffer to be used must be compatible with the protein sample and the detection reagents. For the example above using Cytochrome-C, the buffer to be used is .01M Tris-EDTA, and 2mL of buffer should be placed in each well of the multi-well plate before use.

3. The detection reagents should be used according to the specifications given with the kit purchased. Using the Bradford Assay reagents for Cytochrome-C would require that the standard stock solution be diluted from 10x to 5x stock concentration.

Transport of Protein Across Membranes

1. Insert SepCons™ containing silicon chips with intact membranes with desired pore size into the Nanomembrane Array.

2. Load desired protein sample amount (~100-200ul) into each SepCon™ well. Into one well, load the buffer of interest, and leave 3 wells empty for eventually calibrating the detection mechanism.

3. Assemble the device by attaching the pressure cap and mesh, tightening screws to establish the seals.

4. Using the aligner component, position Nanomembrane Array over multi-well plate.

5. Reconnect the Pressure System (calibrated to desire pressure), and begin applying pressure.

6. Allow pressure to be applied for approximately 15min. taking note of amount of fluid transported so that the membranes do not dry.

7. Remove device from multi-well plate and discard the used SepCons™.

Detection of Protein

1. Add desire amount of detection reagents to the multi-well plates. (Ex. 200ul of Bradford assay solution (5x) to the wells containing Cytochrome-C filtrate in example experiment).

2. Place plate in Tecan Microplate Reader, scanning for absorbance at a wavelength defined by detection reagents (595nm for Bradford Assay).

Pressurization Time

Because our device is intended to filter proteins of wide ranging size, determining a time requirement for pressurization of the Nanomembrane Array to achieve acceptable transport is difficult. While these pncSi membranes have been characterized in terms of H_2O permeability, it is unknown how well this translates to actual molecular filtration applications such as ours. Simple calculations were performed using this H_2O permeability value to quantify the amount of time that would be needed to transport 100µl of water across a 0.4mm^2 pncSi membrane at 2 psi. At a permeability of 10ml/cm^2*atm*min, it was shown that the Nanomembrane Array device would have to be pressurized for just over 18 minutes.[xxvii] Assuming that the presence of proteins in the sample would decrease flow rates to approximately 75% of the H_2O level, we expect that a pressurization time of around 25 minutes would suffice. In order to confirm this preliminary calculation, a significant amount of testing with proteins of varying sizes will have to be conducted. Yet, even with the information from these tests, predicting the time needed to achieve complete fluid transport would be complicated by the variety in efficiency of candidate drugs at breaking large proteins into smaller parts. So, in conjunction with timing

the tests as a means of both achieving complete fluid transport and preventing membrane drying, we made efforts to increase the transparency of our design components, allowing for visual inspection as the test is run.

Evaluation of Solution

Testing Plan

The testing plan that we have outlined is organized by the larger systems that need to be tested, namely the pressure system and the detection system. In order to test the various components of our device separately, as well as the overall system, we proposed the use of a piecewise testing plan that was performed in a particular order. In doing so, we were able to identify potential problems with individual components before testing the overall assembly. Furthermore, testing of individual components made it easier to troubleshoot problems with the overall assembly since we were able to remove variables in which we were confident.

After laying out this testing plan for our device, it became evident that we would not have sufficient time to run all of the tests we would like. As a result, testing was conducted in a logical order, starting with the most basic proof of concept tests. More intricate tests would have to be conducted in the future in order to fully characterize our device's performance.

Results of Testing

Pressure Testing Standards

Pressure testing was a primary focus for evaluating the efficacy of our device. In order to perform proper pressure testing, we referred to ASTM Standard F 37, *Standard Test Methods for Sealability of Gasket Materials.*[xxviii] Although this standard is intended for testing what is effectively a material property of the gasket, rather than testing the seals that exist in a particular setup, it is still a valuable resource by providing guidelines on proper setup, testing, and recording techniques. According to this standard, N_2 is the preferred gas, the leakage rate should be measured by the change in pressure of a water manometer located upstream from the gasket testing fixture, and the results are in the form of leakage rate in milliliters per hour.

Pressure Testing

There are two main components to the testing procedures: testing the ability of the system to hold a pressure and testing the time it takes to pressurize the system from atmospheric pressure.

Pressure Dissipation

The initial goal of our pressure dissipation testing was to find all leaks in our system, and eliminate them. First, we checked the tubing upstream of our design to ensure that there were no significant leaks. Leaks were found using soap and water as well as emersing components in a bath and were temporarily fixed for the purposes of our prototype. A more customized and effective system of tubing would be formulated for future devices.

Secondly, we sealed our Spacer without SepCon™ holes to our Pressure Cap with a pressure feed and the pressure gauge attached, and tested for leaks qualitatively by coating all system interfaces with a soapy water mixture. This pinpointed the specific locations of air leakage, and allowed for further modifications of our design. This test led our group to incorporate four extra bolts for sealing (bringing the total to eight), which proved very effective. We then quantitatively measured the dissipation of pressure by pressurizing the system to a given pressure, sealing off the pressure, then monitoring the pressure over time. The percent of the pressure leaked by a certain time for several different initial loaded pressures are displayed in Figure 13 below.

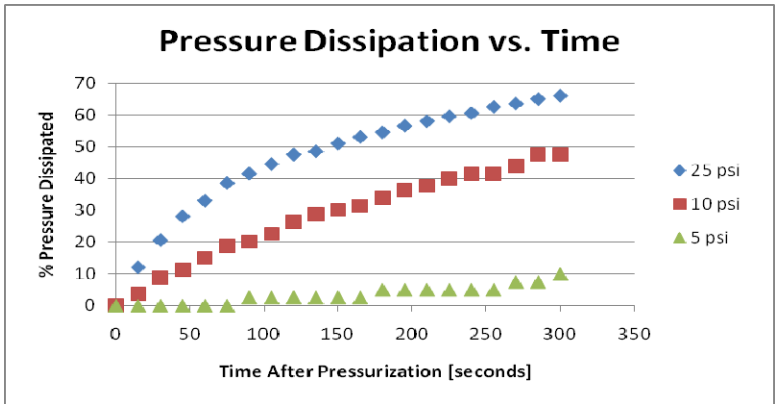


Figure 13: Graph of percent pressure dissipation over time. As seen by the graph, it is evident that the percent leakage in the system grows with the initial pressure obtained, and at low pressures below 5 psi the leakage of the system will be negligible.

After this test we drilled two holes for SepCons™, and reran the tests with SepCons™ in place. It was shown that less leaking actually occurred with SepCons™ in the place, proving that there were no leaks caused by the recently drilled SepCon™ holes. Furthermore, our qualitative testing shows that most of the leaks are extremely small and are caused by supplementary parts of our system, such as the pressure tubing junctions.

Pressure Loading

Another experiment that we conducted, was designed to test the amount of time it took for the Nanomembrane Array device to reach a set pressure after calibrating the device to that pressure. Three trials were run after calibrating the pressure system to establish a pressure of 10psi and 25psi. The observed data showed an increase in pressure from 0psi to the desired pressure according to the equation

$$P(t) = P_0 e^{-\tau/t}$$

Where P_0 is the desired final pressure, τ is the time constant, and t is the amount of time passed since the pressure was first applied. By observing the data shown in Figure 14, a time constant of approximately ~4.5s was observed while trying to attain both pressures. This suggests that it takes 4.5s to reach 63.2% of the desired value. Assuming that after a length of time equal to five times the value

of τ the system has reached the desired pressure, it would take approximately 23s for the system to reach the final pressure. We feel that this is an acceptable value for the time constant as the entire length of time for pressure to run is approximately 15 min, and it would then take only 2.5% of the entire running length to reach the final desired pressure.

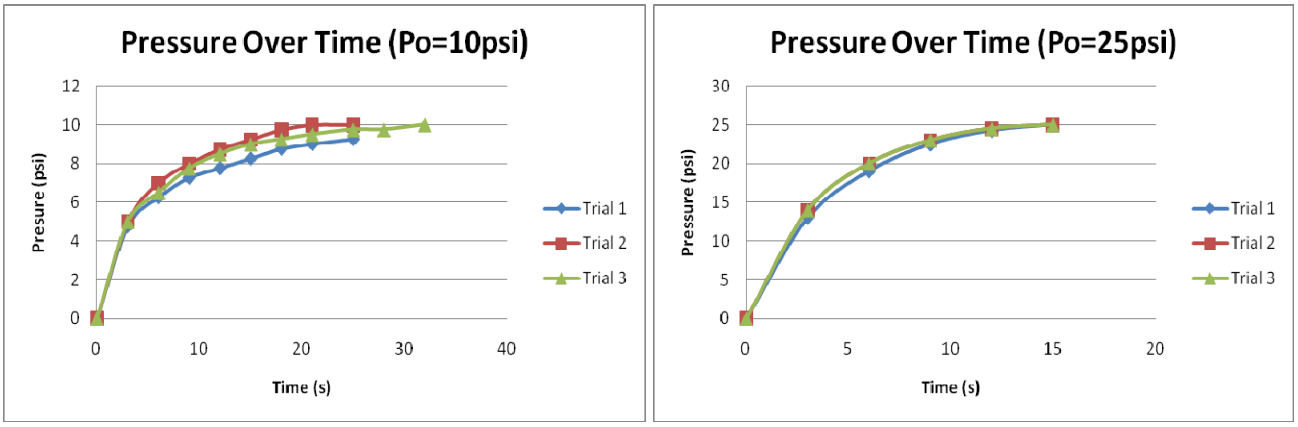


Figure 14: These graphs display the change in temperature within the Nanomembrane Array after calibrating the pressure system to 10psi and 20psi. The time constant for both graphs is approximately 4.5s.

Fluid Transport

As another preliminary test, the Nanomembrane Array device was setup with two SepCons™ in place. These SepCons™ were loaded with equal amounts of dyed water (using food coloring) in order to aid with visual inspection of fluid transport. Additionally, a 24-well multi-well plate was loaded with equal amounts of water in three well locations, with two of these collecting filtrate from the SepCons™ and one serving as a negative control. With the device fully assembled, the Pressure Cap was pressurized to 2psi and maintained there for 15 minutes. After this time period, the Nanomembrane Array was removed from the multi-well plate allowing for inspection of the experimental wells against the negative control well. As displayed by Figure 15, the dye in both of the SepCons™ was effectively transferred to the two experimental wells after the 15 minute pressurization period. The negative control well remained clear. In addition, it was observed that the volumes of fluid in the experimental wells were significantly greater than the volume of fluid in the well containing regular water.

Figure 15: Right, the SepCons™ loaded with yellow and green dye were placed in the Nanomembrane Array device. Left, following pressurization of the device, the filtrate collection wells were observed to change color.

Although this test was fairly simple in nature, our design team feels that it represents vital step forward in the development of a functional device. Effectively, it proves that by establishing a pressure gradient across the pncSi membranes we are able to cause significant fluid transport. While this test does not introduce the complexities of transporting peptides, it will serve as a starting point from which more rigorous testing can go forward.

Dilution Testing

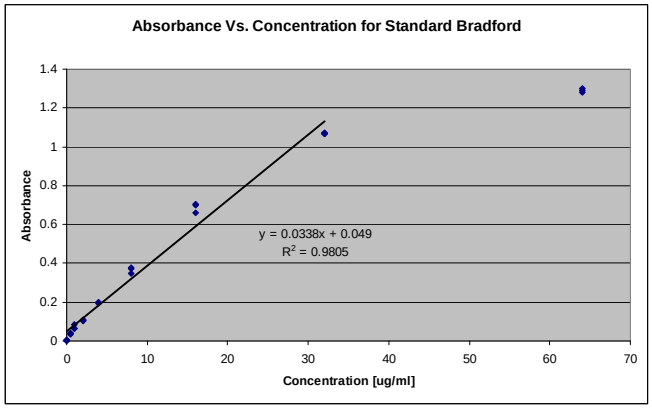
The goal of the primary dilution testing that we have done is to determine if a Bradford assay is a suitable method of quantifying filtrate concentration for the projected usage of our customer. Detection system are primarily restricted by the dilution of the samples during filtration across the pncSi membranes.

For high throughput testing, it is desirable to conserve as much of samples as possible. However, as our prototype device currently exists, the underside of the membrane needs to be wet, and therefore the well underneath each SepCon™ needs to be filled with a significant volume of buffer. With our prototype, this buffer-membrane contact occurs when a volume of 2 ml of buffer. According to specifications given to us by Dr. Harold Smith, it is cost effective to use 100µl of between 0.5 and 3 mg/ml hA3G in each well. There is also a slight difference needed to be accounted for due to the fact that there must always be a small excess of fluid remaining on the upper side of the membrane to ensure no drying and cracking, resulting in approximately 20µl of wasted sample. Although this results in a 26 times dilution in our prototype, in a projected 96 well format, this would only be a 3.48 times dilution, and, given elongation of the SepCon™ design, would be even less. The final concentration of the APOBEC3G sample will be between 19.2 and 115µg/ml for fully reacted APOBEC3G loaded and filtered perfectly from each donor well.

Detection Tests

As noted before, the goal of the detection testing was to find an assay technique that is potent enough to make detection readings at probable filtrate concentrations. For our prototype, these values range from 0 to 115µg/ml depending upon drug candidate efficacy.

Initial testing of the Bradford detection system began with an analysis of the linear range of the system with respect to our device. This constituted ensuring a negative control is present in the samples, as well as finding a linear range of the detection system. The reagent used for this was a 5x diluted Bradford reagent from Bio-Rad Laboratories, Inc., as provided from Dr. McGrath's lab. Because this is a test of absorbance, both the concentration and the pass length (the depth of liquid in the well) altered the readings. Therefore, we needed to calibrate the testing to work with 2ml of total final sample in each well. We received the following normalized data for a standard concentration of Bradford reagent added.

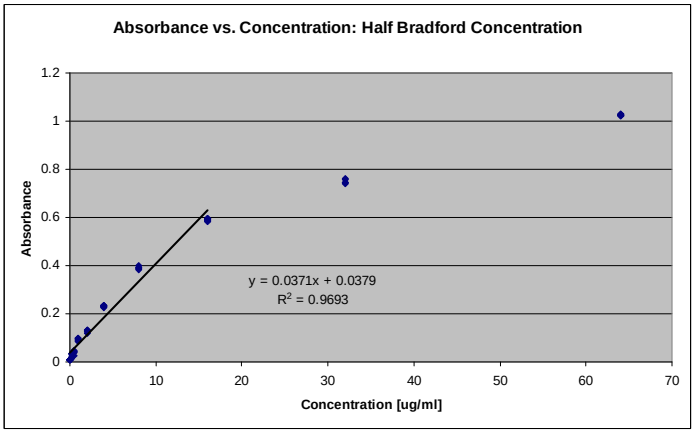


Conc. (µg/ml)	Run 1	Run 2	Run 3	Average
H_20	0	0	0	**0**
0	1E-04	0.0035	0.005	**0.002867**
0.5	0.0354	0.0391	0.0376	**0.037367**
1	0.0852	0.0657	0.0629	**0.071267**
2	0.1078	0.1067	0.1038	**0.1061**
4	0.1984	0.2006	0.1928	**0.197267**
8	0.3767	0.3732	0.3484	**0.3661**
16	0.7049	0.6995	0.6618	**0.688733**
32	1.0746	1.0668	1.0695	**1.0703**
64	1.2802	1.289	1.2992	**1.289467**

As displayed by the data above, a difference exists between the negative control buffer and the zero of water by less than 0.01 absorbance index (which ranges from 0 to 1). Upon conduction of multiple linear regressions, it becomes evident that the absorbance follows a linear range from the lowest tested values to 32 µg/ml concentration of BSA. However, as absorbance values rise above 1.0, the scale is not reliable. Therefore, subsequent tests were performed with lesser concentrations of Bradford reagent as displayed below.

Conc. (µg/ml)	Run 1	Run 2	Run 3	Run 4	Run 5
H_20	0	0	0	0	0
0	0.0089	0.0066	0.0074	0.0086	0.0079
0.125	0.0107	0.0137	0.0143	0.0143	0.0139
0.25	0.0227	0.0281	0.035	0.0338	0.0349
0.5	0.0248	0.0392	0.0424	0.0451	0.0431
1	0.0836	0.0913	0.0942	0.0965	0.0975
2	0.1201	0.128	0.1287	0.13	0.1317
4	0.227	0.2319	0.2324	0.2322	0.2294
8	0.399	0.393	0.393	0.3874	0.3841
16	0.5938	0.5964	0.5926	0.586	0.581
32	0.7625	0.7435	0.7394	0.7458	0.7594
64	1.0246	1.0209	1.0237	1.0272	1.0296

Projected Maximum	R-squared
0.25	0.7733
0.5	0.7287
1	**0.9608**
2	**0.9179**
4	**0.9582**
8	**0.9686**
16	**0.9432**
32	0.8502
64	0.8055



This data, along with the R-squared values for associated linear regressions, shows that the optimal range of the Bradford assay is from 1µg/ml to 16µg/ml, which is acceptable for the dilutions that our customer will be working with.

Future Testing

Subsequent Pressure Testing

Future testing is still required for final marketability of our device. The magnitude or duration of the pressure needed to drive proteins through the membrane is currently unknown, as is the concentration of proteins that can be run through the membranes without clogging the pores. Our final goal of this testing is to achieve a protocol for our device that minimizes the sample concentrations needed to be run as well as minimizing the time needed to run each test.

Another important measure to investigate is the pressure dissipation from intact membranes in comparison to those with blown out pores. This value, if great enough, could allow for our protocol to include an detection of failed membranes step, thus making the test invalid. It is also important to investigate the pressure limit to break membranes so that our protocol does not exceed this value. To

do these more exact experiments, it will most likely be necessary to find a new, higher quality pressure regulator. The Narishige microinjection pressure controller, although acceptable for prototyping, introduces a great amount of variability into our tests.

Subsequent Detection Testing

Further tests will also be needed to prove that our device is compatible with a detection system beyond simply finding a linear range. Also, we will have to determine the effectiveness of protein transfer across the membrane as well as the specificity of the membranes for transfer.

The first test that we have devised would determine how freely the small protein moved across the membrane. This will be a test in which we load several different concentrations of low molecular diameter proteins into our device, and with a specific protocol for running time and pressure intensity, we will determine the amount of protein that is transferred compared to the theoretically perfect permeability model. This can be explained by the following equations for flow percentage, where the protein and buffer can be calculated from initial and final concentrations.

$$[Filtrate] = \frac{n^{*}_{\text{filtered protein}}}{V^{*}_{\text{filtered buffer}}} \; ; \; [Sample] = \frac{n_{\text{initial protein}}}{V_{\text{initial buffer}}} \; ; \; \text{Flow } \% = \frac{[Filtrate]}{[Sample]} * 100$$

This test would be performed with Cytochrome-C or Sodium Fluorescein because they are lower molecular weight than BSA, and it is easier to create membranes to fit their specifications. Optimally, we would like the final flow ratios to be as close to 1.0 as possible, but it is projected that there will be a caked area of proteins on the upper side of the membrane due to pore clogging.

Further tests will then be needed to determine the effect of adding a larger diameter protein to the sample as these will always be present during our customer's trials. Initially, the correct pore size membranes will have to be determined through iterative testing by finding the lowest diameter pncSi membranes that effectively block these larger proteins. Following this, we will test to determine whether these large proteins have any effect on the transport of protein through the membrane. This can possibly be modeled by a proportion of the flow percentage of the sample versus that of the control. Our goal for this is to maximize this proportion with respect to pressure intensity and duration. This test would still be used with Cytochrome-C or Sodium Fluorescein with an addition of either IgG or β-galactosidase.

Cytochrome-C Testing

The purpose of this testing plan is to test the ability of a protein molecule to be filtered through an intact membrane and to then detect the protein using a Bradford Assay. The protein to be tested is Cytochrome-C and the testing is intended to be completed with the current prototype (containing two SepCon™ holes).

Calibrating the Pressure System

6. Insert SepCons™ containing blank silicon chips (silicon wafers with no membranes) and assemble device, tightening screws to establish the seals

7. Connect Narishige Pressure Device to the Nanomembrane Array via the Pressure Connector. ***Note: Be sure that the initial flow is set to 0!!
8. Slowly apply pressure using balance until the desired pressure is reached and maintained within the cap. In this case, the pressure will be calibrated to 2 psi.
9. Lock the balance knob in order to preserve the level of pressure.
10. Remove Nanomembrane Array from the Narishige Pressure Device.

Preparation of Protein Samples/Buffers

Cytochrome C

1. Dilute Cytochrome-C from stock (10mg/mL) to desired concentration of 16ug/mL (max of linear range for Bradford Assay)

Buffer (in Multi-well plate)

1. Fill 2ml of .01M Tris-EDTA buffer into the wells of the Multi-well plate to be used.

Bradford Assay

1. Use stock solution, will be using half the standard (5x solution vs. 10x solution).

Transport of Protein Across Membranes

1. Insert SepCons™ containing silicon chips with intact membranes into the Nanomembrane Array
2. Load 200ul of Cytochrome-C solution (16ug/mL) into one SepCon™ well; Load 200ul of Buffer (.01M Tris-EDTA) into another SepCon™ well.
3. Assemble Pressure Cap, tightening screws to establish the seals.
4. Load 2ml of .01M Tris-EDTA into the plate wells into which the SepCons™ will be placed
5. Using aligner, place Nanomembrane Array over Multi-well plate.
6. Reconnect the Pressure System (calibrated to 2psi), and begin applying pressure.
7. Allow pressure to be applied for a range of times (~3-10min), taking note of amount of fluid transported.

Detection

1. Add 200ul of Bradford assay solution (5x) to the wells containing the filtrate
2. Place plate in Tecan Microplate Reader, scanning for absorbance at a wavelength of 595nm

Future Development

The development of our prototype has been largely an effort at a proof of concept for an array that integrates the pncSi membranes for HTS purposes. As such, a large focus throughout the project and at each level of design has been the consideration of future changes, improvements, or opportunities. Future initiatives will seek to increase the marketability of the Nanomembrane Array, make it more compatible for HTS, lessen the operating time and number of components of the device, and allow for mass manufacturing.

Limitations of Current Prototype

A number of limitations have been identified for our prototype device. These would be some of the first issues addressed when going forward with future versions.

The custom-made components for our current prototype were machined using equipment readily available to members of the Biomedical Engineering Department at the University of Rochester. However, the time to complete all of the necessary components was greater than 10 hours. As such, this manufacturing method would be very costly and time consuming if the device was to be marketed. Also, the prototype Nanomembrane Array is composed of many separate pieces; including the Pressure Cap, Spacer, Mesh, SepCons^{TM} (and associated components), outer gasket, and o-rings. This poses a dilemma because in order for the necessary air-seals to be formed, each piece must interact optimally with the others. This requires low machining tolerances when manufacturing the device. Slight variations in the thickness of materials and dimensions after machining could result in a pressure leak as the gasket will not completely fill the gap. In the most important cases, the machining was conducted within a 1/1000 inch tolerance. The dilution of filtrate is another crucial concern, as currently both sides of the membrane must be wet in order for fluid to flow. If the amount of protein is not diluted evenly or is diluted too much for the detection to accurately measure the amount of protein then the device will not function accurately. Further research into the flow rates of filtrate through the pncSi membranes must be explored for proteins of varying size and charge. This will aid in developing a target magnitude of pressure differential and in defining the length of time for the pressure to be applied. The length of time the pressure is applied is especially important because if it is applied for an extended period of time, the top side of the membrane may become dry. As mentioned previously, in the event that a membrane becomes dry on one side, the likelihood of failure and membrane cracking is greatly increased.

Future Design Changes / Marketing Improvements

Scale-up to 96- or 384-well Format

A major goal of the Nanomembrane Array is implementation of the device as a tool in HTS. The prototype we have developed interacts with a 24-well plate design, but in order to be competitive with current high throughput technologies it would need to be scaled up to align with a plate containing 96- or 384-wells. These are standard multi-well plate sizes, and by designing our device to work with common laboratory equipment the number of custom parts required is reduced. Such scale up will allow the users of the device to conduct experiments with a much greater amount of samples. It will also provide a mechanism to traverse libraries of drugs with tens of thousands of candidates in a practical amount of time.

The only major changes that would have to be made to our device's components in order to enact this scale-up would be the redesigning of a Spacer with more holes and the creation of a corresponding Mesh.

SepCon™ Redesign

In order to scale our current prototype to a plate with a greater number of wells, another crucial step is redesigning the SepCons™ that house the membranes. Currently the SepCons™ are made of stock components provided by SiMPore, Inc., and as such the prototype has been developed to incorporate the SepCons™ as defined by available standards. Redesigned SepCons™ will encompass two new traits. First, they will have a smaller diameter than the current design, so as to allow them to fit comfortably in the smaller wells of higher-throughput plates. The current SepCon™ models are only true compatible with the 24-well plates as a result of their bulkiness. In addition, the length of SepCons™ would be increased to allow the membrane to be positioned lower in the well. This is largely advantageous because the amount of buffer in the well could be reduced, and as such the dilution of the filtrate could be minimized.

SepCon™ Assembly / Dispensing

The use of SepCons™ imposes a significant time constraint on the set-up of the device. It is impractical to expect the user to load each SepCon™ individually during a HTS operation. Therefore, a method for loading and removal of the SepCons™ quickly and accurately is required. SepCons™ inherently have a time requirement for assembly, and the assembly process requires careful attention in order to keep the membrane intact. To overcome these obstacles, the SepCons™ will be pre-assembled either as part of our manufacturing process or by an outside company (most likely the SepCon™ producer) and will then be distributed in a safe, sterile manner that will protect membrane integrity. Also, the SepCons™ will be delivered in conjunction with a device similar to those used to dispense standard pipette tips from their packaging into their respective sterile containers. Essentially, the SepCons™ will be positioned on an array, likely made of plastic, which will allow them to be positioned correctly into the device simultaneously. Inserting the SepCons™ will thus be a parallel process, and removing them could follow a similar procedure using a device that can remove each SepCon™ concurrently. This process will also minimize the handling of the membranes by individual users of the device, lessening the chance that the integrity of the membranes will be sacrificed.

SepCon™ / Spacer Integration

A more elegant solution to the issue of increased set up time that accompanies the use of SepCons™ is the integration of the pncSi membrane directly into the Spacer. This would eliminate the need for individual membrane holders (SepCons™). The Spacer would need to incorporate several of the characteristics of the SepCons™, including a mechanism for safely positioning the membrane, a large enough reservoir to maintain the samples without cross contamination, and individual projections for each membrane would be required to fit each well. This is highly advantageous as it will remove the requirement that each SepCon™ must generate an airtight seal between itself and the Spacer. The Spacer would no longer be reusable, however, and each time the experiment is conducted a new Spacer would be necessary. This would be far too costly if the construction of the device required delicate machining, and would only be feasible in the event that the entire Spacer was produced by injection molding.

Mesh / Pressure Cap Integration

The current prototype utilizes the interaction of the pressure cap and mesh to apply the necessary force to each SepCon™ to form a seal and to allow the pressure differential to be applied to each SepCon™. Each piece was machined independently, but each must fit together in a preferential manner and aligning the pieces during assembly is a process that requires careful attention. As such, it is likely that these pieces will be merged into a single cap that has the capability of applying pressure to each SepCon™ individually. The mechanism of applying pressure to the SepCons™ will need to distribute the force evenly while allowing the transport pressure differential to be established in each SepCon™. Likely, projections of material will descend from the top of the cavity in the cap and extend to the top of the SepCon™. These projections will also incorporate a network of channels of removed material that is connected to the pressure source. Machining such an intricate design into our prototype would have been timely and too costly, but future manufacturing that could incorporate automated machining or injection molding would provide a realistic mechanism to integrate these two components.

Improved Clamping Mechanism

An essential aspect of the Nanomembrane Array is a means of applying pressure to the Pressure Cap and Spacer in order to firmly force them together. The clamping system for the prototype has proven to be capable of establishing the airtight seals and maintaining a pressure differential. This involves the use of 8 bolts spaced along the outside edge of the Spacer. These bolts fit into particular threaded holes in the Spacer. The Pressure Cap provides channels through which these bolts pass, and wing nuts provide a mechanism to establish the pressure. While this design is successful, future considerations may focus on developing a clamping system that is ideal for high throughput processes. In other words, any useful future developments would need to generate the required airtight seals while reducing the amount of time to activate the clamping mechanism. For example, a device that applies pressure evenly to a metal plate that aligns over the device would accomplish the necessary forces to generate all of the airtight seals. This mechanism would likely incorporate a means of force that would require less time to assemble as it would hopefully only need to be tightened at one position, rather than eight positions.

Improved Pressure Regulation / Supply

One of the key external interfaces of the Nanomembrane Array is a pressure system that can establish the pressure differential for transport. The developed prototype has been designed to interact with the Narishige Pressure Device. The pressure system made by Narishige allows pressure to be applied continuously through a balance module or injected though an injection module that is activated by a foot pedal. While this system is capable of establishing, maintaining, and editing the desired pressure, it has no inherent mechanism for measuring the pressure being applied. The pressure that is output is subject to the pressure input, which is an issue since the input pressure varies in the prototype system due to variability at the source. The current pressure system must be calibrated each time a new pressure differential is desired or when the input pressure is altered. Future commercial pressure systems that could interact with our device should have a better means of measuring the output pressure. Ideally, the pressure system would incorporate a method to intake the desired pressure

output as an input, and thus calibration of the device would not be necessary for each trial. It is likely that a pressure gauge would remain integrated into the pressure cap as a means of supervising the actual pressure within the device.

CO_2 Cartridges

Since not every biochemistry lab may have a nitrogen tank or air supply and regulator on hand, we have spent some time considering other possible options for pressurizing the system. One idea that we had in mind was to use disposable CO_2 cartridges, like those used to repair a flat bike tire in an emergency. They are small, lightweight, and easily carried in the pocket of a cyclist on a long ride. An example of a CO_2 cartridge can be seen in Figure 16.

Figure 16: Cartridges such as the one displayed above would be modified to contain N_2 gas rather than CO_2.

These cartridges typically come in 12g and 16g sizes, and are capable of quickly inflating a road bike tire to 90-125 psi. They can be ordered in bulk (500 cartridges) at a cost of about $.50-$.60 per cartridge, making them a relatively inexpensive, “throwaway” part.

By placing the appropriate fitting on the top of our pressure cap, the user could simply press the cartridge into place for a few seconds to quickly pressurize the system, then walk away. Since we have shown that our device is currently capable of maintaining pressures in the desired range without the assistance of balance air flow, the initial fill would be the only required interaction by the user. The system could sit on the lab bench, dissipating pressure slowly through the membranes, while the user performs other tasks.

This solution poses one major drawback, however. Since the volume of a bike tire is significantly larger than the volume of our cap, the pressure that is generated would be much too great for the membranes to withstand. After performing some calculations that take into account the volume of the pressure cap (4.9 in^3), we found that a 12g CO_2 cartridge would pressurize the cap to about 854 psi. Since we plan to work with pressures on the order of about 2 psi, this is obviously too high.

While we acknowledge that using 12g CO_2 cartridges is not currently a viable solution, the concept is highly attractive. It is possible that smaller cartridges may be purchased, or that a future version of our device would have a large enough chamber to allow for their use. Since the cartridges are relatively inexpensive and remove the need for each laboratory to have the previously required pressure equipment, they are worth pursuing in future versions of our device.

New Fluid Transport Mechanism – Electroosmosis

As mentioned previously, the use of electroosmosis as a means of transporting materials across the membrane had several drawbacks that steered us away from its use during our prototyping phase. However, electroosmosis would provide a very elegant solution once the current technical issues have been resolved, namely pH and temperature changes at the electrodes.

In order to adapt our array to use electroosmosis, we would need electrodes that extend down into each sample, and also have electrodes placed in the bottom of each well of the multi-well plate. We envision a mesh of electrodes made out of metal (either Au or Pt) that would be placed over the top of the SepCons™ (or re-designed SepCons™), with one electrode extending into each SepCon™. This can be seen in Figure 17.

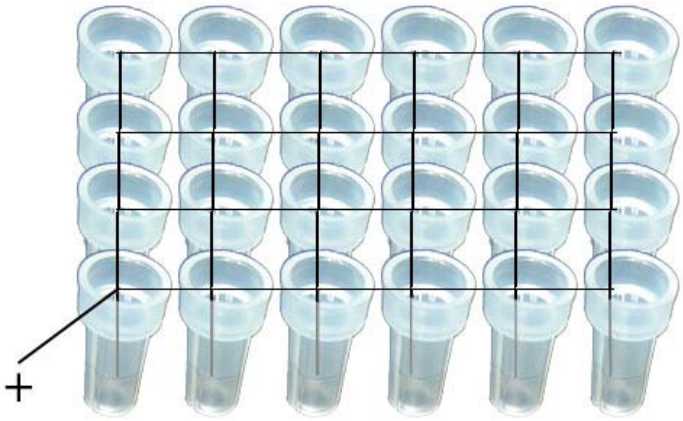


Figure 17: An array of SepCons™ with positive electrodes (anodes) inserted.

In order to induce flow, we would also need to insert electrodes into the liquid in the wells of the multi-well plate. Again, a mesh of electrodes would be placed over the multi-well plate, with one electrode extending into each well. This can be seen in Figure 18.

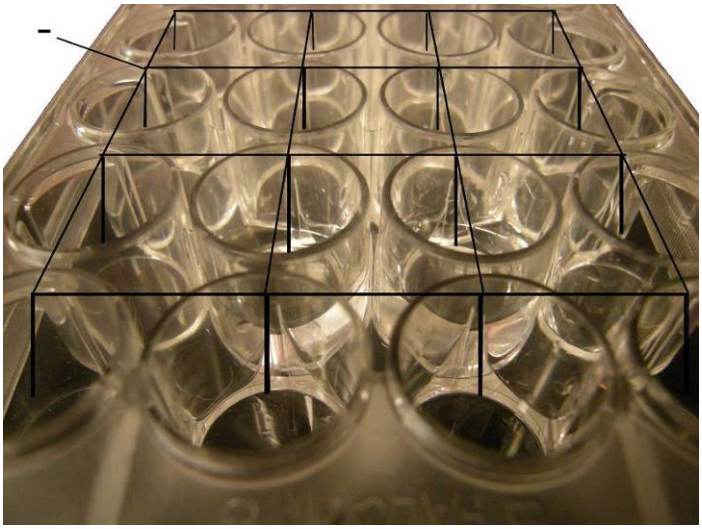


Figure 18: The filtration collection zone (multi-well plate) with negative electrodes (cathodes) inserted.

With both of these electrode meshes in place, opposite charges can be applied to the liquids on either side of the membrane. This would greatly simplify our design, since a pressure controller, bulky pressure cap and Spacer, clamps/screws, and concern about pressure seals would be removed. As you can see, this is a very minimal design, but maintains the benefits introduced by use of the novel pncSi membrane material.

Completely Automated Process

The prototype developed and the future advances previously described are for a device that would be utilized primarily by academic research efforts or research with limited budgets. If the concept of drug screening with the Nanomembrane Array were to reach the level of testing used by profit driven drug companies, it is likely that each aspect of setting up and running the device would be automated. This would require the development of an automated machine that could follow the protocol for running the device. Each component of the device would need to integrate a method for interacting with the automated system. Transport times in such a design would need to be well documented or a mechanism for monitoring them would need to be developed as a measure to prevent the drying of membranes. Automating the processes for loading and detecting the presence of protein are already incorporated into our device, but the process of switching between each step must be automated. While such a project is not unfeasible, it would be far too costly unless undertaken by an organization with adequate funds such as a pharmaceutical company.

New Manufacturing Techniques – Injection Molding

As has been discussed, several future endeavors would benefit greatly or require a new source for manufacturing. The current methods, primarily machining, were time and cost intensive, and the total cost of the prototype device alone was greater than $700. Machining provided a method to create the device with low enough tolerances that it would be able to seal pressure. However, if the device were to move past the prototype phase into a mass manufacturing phase, injection molding would be the likely route to construct individual devices. Such a process incorporates a very large input cost, the

cost of creating a mold out of metal or alloy, which will then be used when making an individual component. The benefits of this process are that each individual component made is extremely cheap, which is why this form of manufacturing is only practical in a mass manufacturing scenario. Also, injection molding will allow a more intricate design of the components, likely reducing the number of parts to two, a combined pressure cap and mesh, and a combined Spacer and SepCon™. There are some inherent defects involved with injection molding, such as greater tolerances and defects in the device, especially around the area where the two sides to the mold clamp together. By reducing the number of "soft spaces" required for gasket materials and increasing the depth to which the membranes sit in the multi-well plate reduce the need for tight tolerances and would further support the use of injection molding.

Realistic Constraints

In addition to the design improvements that would be incorporated into future versions of our device, our design team identified a number of realistic limitations that we would likely have to address prior to further development or marketing. These "realistic constraints" on our design included manufacturing processes, manufacturing economics, the economics and implementation of HTS systems, and pertinent laboratory safety procedures. For access to the reports on these topics please contact Nanomembrane Array design team members.

Design Process Evaluation

Lessons Learned

Throughout the design process, our group has learned several valuable lessons. One of the most important of these lessons is the need for constant reflection on the overall goal or problem to be solved, as well as constant revision of certain aspects of the design. Constant revision of the problem statement allowed us to incorporate new information, new knowledge, and more focused understanding into key design decisions.

A perfect example of the value of revision was the decision to change the interface between the SepCons™ and the Spacer. Originally, each hole in the Spacer was to have a small groove machined around it. An o-ring would sit in the groove, a washer would sit on top of the o-ring, and the SepCon™ would pass through both. This design detail was based on the desire to closely regulate the depth of the SepCon™ below the Spacer. We felt that the small degree of variability inherent to the deformation of the o-ring might be enough to upset the necessary dilution of samples in the multi-well plate. However, after learning more about the particular detection system a user may employ, we found that we did not require as precise placement of the SepCon™ as we had originally thought. This meant that we could remove the washers and grooves from the Spacer, significantly reducing both the complexity of our design and the required machining.

Another important lesson our group learned during this process is that our device required longer machining time than we had originally predicted. In our previous design reviews, we had

underestimated the time required to machine parts as we designed them. Although the parts themselves may be very straightforward and relatively simple, the process of machining them may not be so easy. Time is required to set up a job, carefully place the parts, and slowly move the drill into place, not included in our initial preconceptions. This was an important lesson to learn, since it led to a better understanding of the future manufacturing options for our device. The time and cost associated with machining make injection molding very attractive, as discussed previously.

Finally, our group learned the importance of performing some preliminary evaluations of things that may seem like common sense. These evaluations can be as simple as back-of-the-envelope estimates or very basic tests that may not seem important but should be performed. For example, we had hoped our ¾" polycarbonate Spacer would not bow in the middle when any of the sides were compressed, but it did very slightly. Had we spent time looking at a solid mechanics problem, we may have predicted this outcome. Luckily, we were prepared to solve the problem when it arose by increasing the number of clamps around the device.

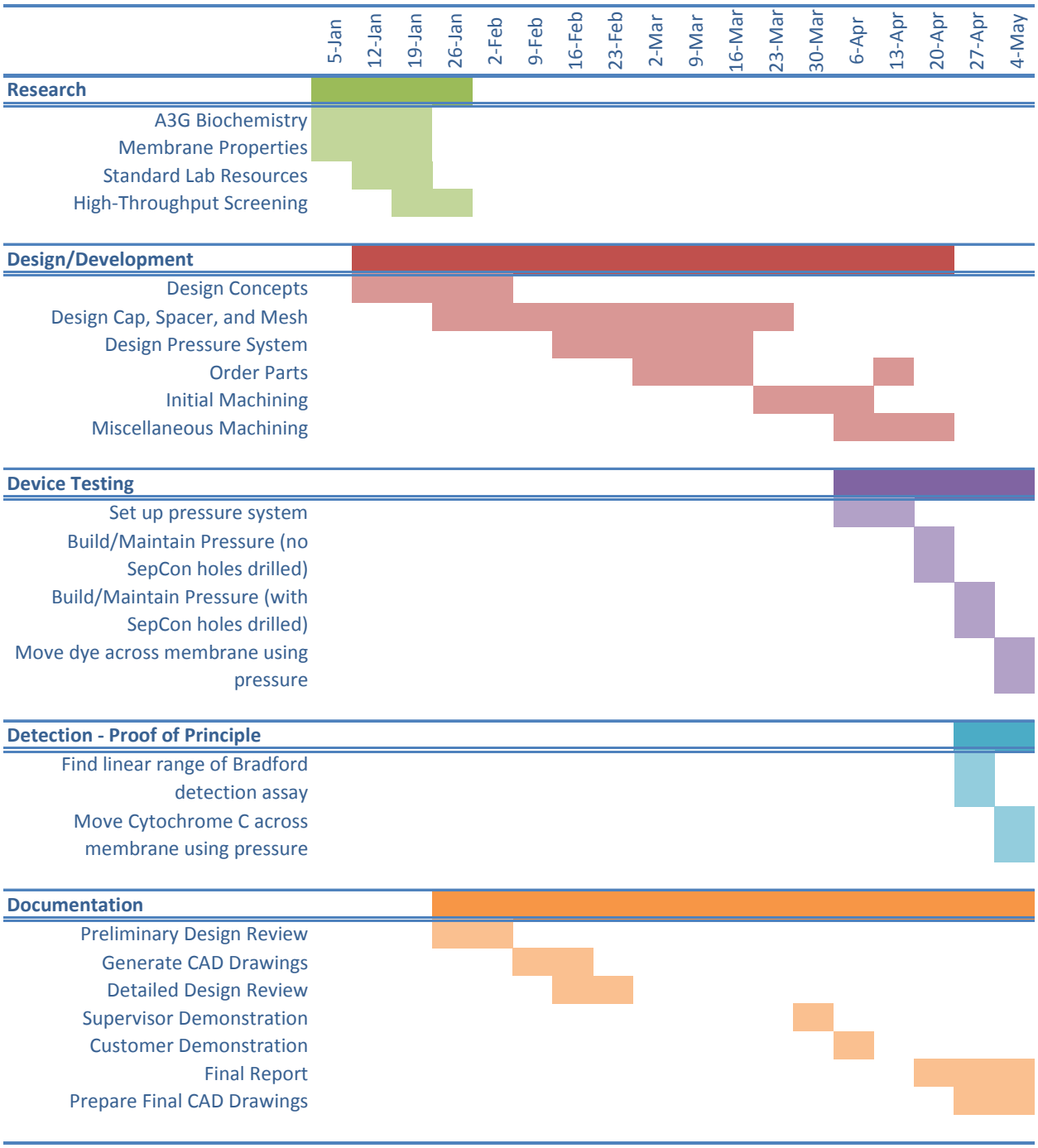
Budget

In order to complete the prototyping phase of this project, a budget of $600.00 was allocated to be used to supply any materials, machining, or chemicals to successfully build and test the device. Through generous donations from Dr. McGrath's lab and Dr. Harold Smith, many of the required components to build and test our device were donated, and as such were not taken into consideration when calculating the $600 budget. As far as materials and equipment that were budgeted into the allocated $600, we were able to keep the cost low at approximately $233. However, we were supposed to factor in the cost of machining, but because our device required many intensive machining hours, the overall cost of machining was around $675. This itself is over the allowed budget, and when considered into the actual budget, our group spent a total of around $1042, a total of $442 over the theoretical budget amount.

Actual Purchases		
Item	**Cost**	**Balance**
12"x12"x1/4" Acrylic Sheet	$9.15	$590.85
12"x12"x3/4" Polycarbonate Sheet	$81.41	$509.44
0-5 psi Pressure Gauge (x2)	$91.04	$418.40
Washers	$1.53	$416.87
O-Rings	$2.97	$413.90
O-Rings	$6.00	$407.90
Nuts/Bolts/Wing Nuts	$6.79	$401.11
Gasket	$14.89	$386.22
Standards Package	$10.00	$376.22
Metric Tap/Screws	$8.50	$367.72
Total	$232.28	$367.72

Donated Parts/Materials/Labor	
Item	**Estimated Cost**
Tecan Infinite 200 Series Plate Reader	$29,000.00
Janus Automated Workstation	$40,000.00
Narishige Microinjection Pressure Controller	N/A
Multi-well Plate (x2)	$95.45
Tubing (.17"Idx.25"OD)	$7.52
SepCons with membranes (x10)	$100.00
PDMS (partially used)	$11.69
Machining (15)	$675.00
Buffer (Tris-EDTA)	$71.80
Bradford Reagent (500mL)	$47.60
Protein (BSA-250mg)	$34.00
Total	$70,043.06

Design Schedule



Conclusion: Does it work?

The question of whether or not our device "works" is difficult to answer. On some levels, our device was absolutely a success, while on others, it merely marks a proof of concept stage in a much longer design process.

For the purposes of this year-long design and prototyping process, we laid out a few major goals. We hoped to design a device that could effectively incorporate SiMPore, Inc. pncSi membranes into an array format, subsequently move material across the membranes by way of a transport mechanism, then detect the presence of a particular protein in the filtrate. Through our pressure testing, we were able to show that we did successfully construct a device that can build and maintain operating pressures with little to no dissipation, as well as build and reasonably maintain pressures more than an order of magnitude greater than necessary for operation. Through our dye test, we showed that we did successfully construct a device whereby material could be moved from one side of the membrane to the other using a transport mechanism, since the colored dye passed through the membrane and tinted water that was originally clear. Finally, through our detection testing, we showed that we did successfully construct a device that was dimensioned such that the resulting filtrate would be of proper concentration to be detected by a common assay.

Looking at the overall picture, we still feel that our device is successful. While we are not able to screen candidate drugs at a pace of about 1000 per week, we were able to produce a device that can be used by a biochemist performing testing as a means to increase their throughput. This device, in its current state, can be used in orthogonal, second-level screening of initial hits, but it does not compete with fully automated, highly specialized high-throughput screening setups.

With that being said, it is important to note the significant steps that were made in providing substantial proof of concept for the eventual integration of pncSi membranes into a high-throughput screening setup. We were able to show that these membranes could be used to separate proteins based on size, and that a pressure system could be used to quickly transport a large number of samples simultaneously across membranes, but a large amount of future development is necessary before the device will meet current high-throughput needs. We have spent significant effort during our design process focusing on the aspects of our project that contribute to its future development and have shown that the infrastructure is in place to easily scale up our system. Each important design decision was made with future development in mind.

Acknowledgements

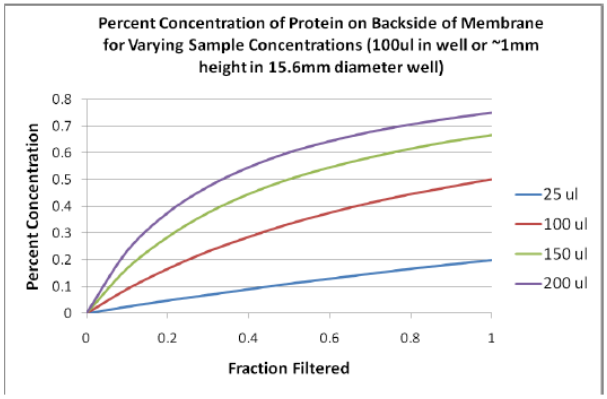
Certain members of the University of Rochester community have been invaluable to the completion of this design process. The Nanomembrane Array design team would like to express our sincere gratitude for the contributions made by Art Salo, Rachel Twardowski, Barrett Nehilla, Henry Chung, and Jessica Snyder; and, also to our team supervisor, Dr. James McGrath, and Dr. Harold Smith for their guidance throughout.

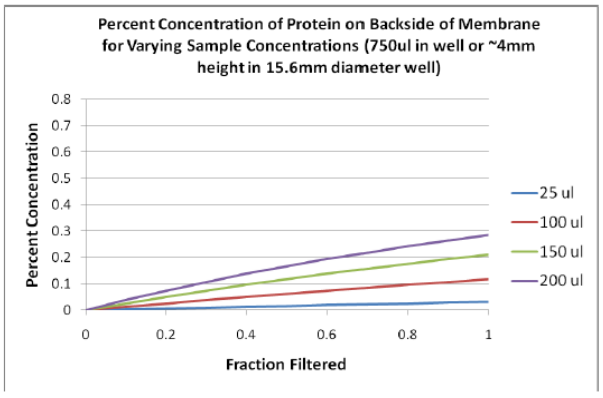
Appendices

Appendix A

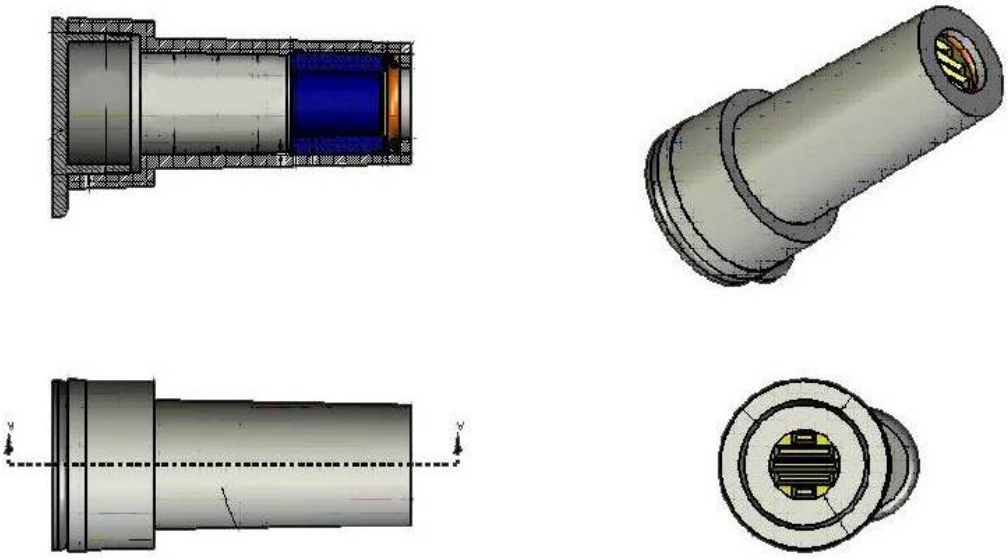
	Categories	Prevents Contamination	Compatibility with Pressure System	Membrane Reliability	Number of Parts to Design/Machine/Buy	Ease of Filtrate Collection	Cost	Speed/Ease of Trial Setup	Elegance of Design		
Membrane Integration Designs	Weights	15	15	15	10	10	10	10	5	Total Score	Ranking
SepCons (SiMPore, Inc.)	Scoring	8	7	8	4	9	5	3	4	575	1
Full-wafers	Scoring	3	4	3	9	5	7	9	8	490	2
Multiple-nanomembrane Chips	Scoring	3	2	6	6	5	6	6	7	430	3

The decision to use SepCons™ has thus far been justified by qualitative factors more so than by models or calculations. However, as was mentioned previously, SepCons™ afford us greater control over dilution than our other concepts for pncSi membrane integration. With this important design factor in mind we have begun compiling preliminary dilution calculations based on the depth to which the SepCon™ is placed inside our filtrate collection zone. Graphs for a few potential scenarios of this model are provided below. For various volumes of sample and buffer, dilution of filtrate is calculated on a continuous scale as a greater percentage of sample is moved across the membrane.





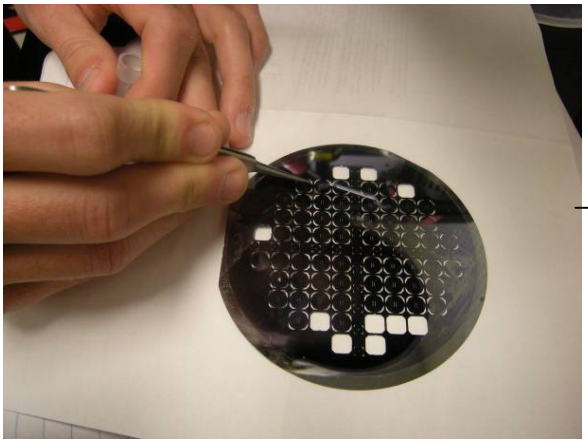
Appendix B



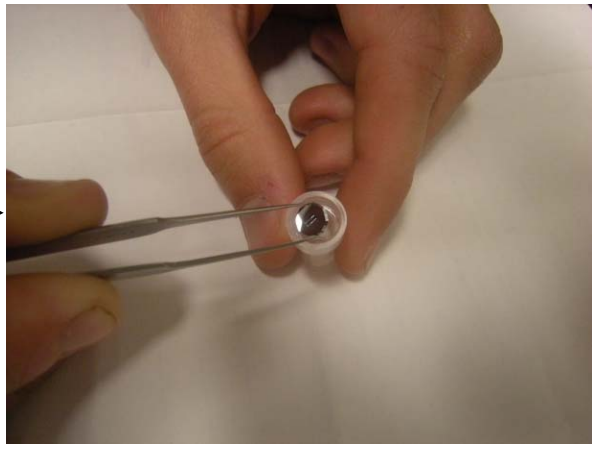
Appendix C

SepCon™ components and basic assembly procedure:

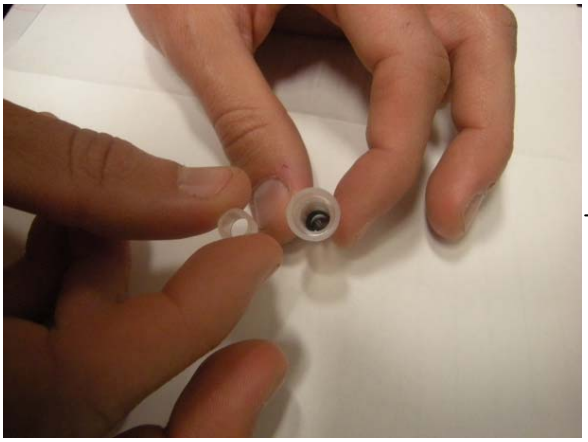
Three components of the SepCon™ are displayed above. From left to right, they are the housing cylinder, inner securing cylinder, and o-ring. The nanomembrane is not shown.



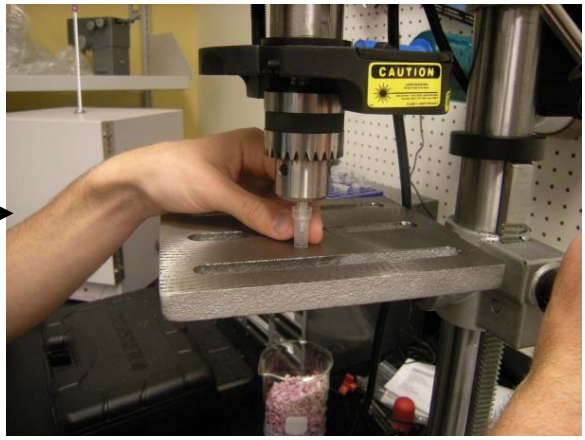
1) The individual nanomembrane piece is removed from the silicon wafer



2) The o-ring and nanomembrane pieces are then inserted in the correct orientation to the bottom of the cylinder



3) Next, the inner securing plastic piece is placed in the bottom of the housing



4) With all three pieces inside the housing, a drill press is used to compress and create a pressure tight seal

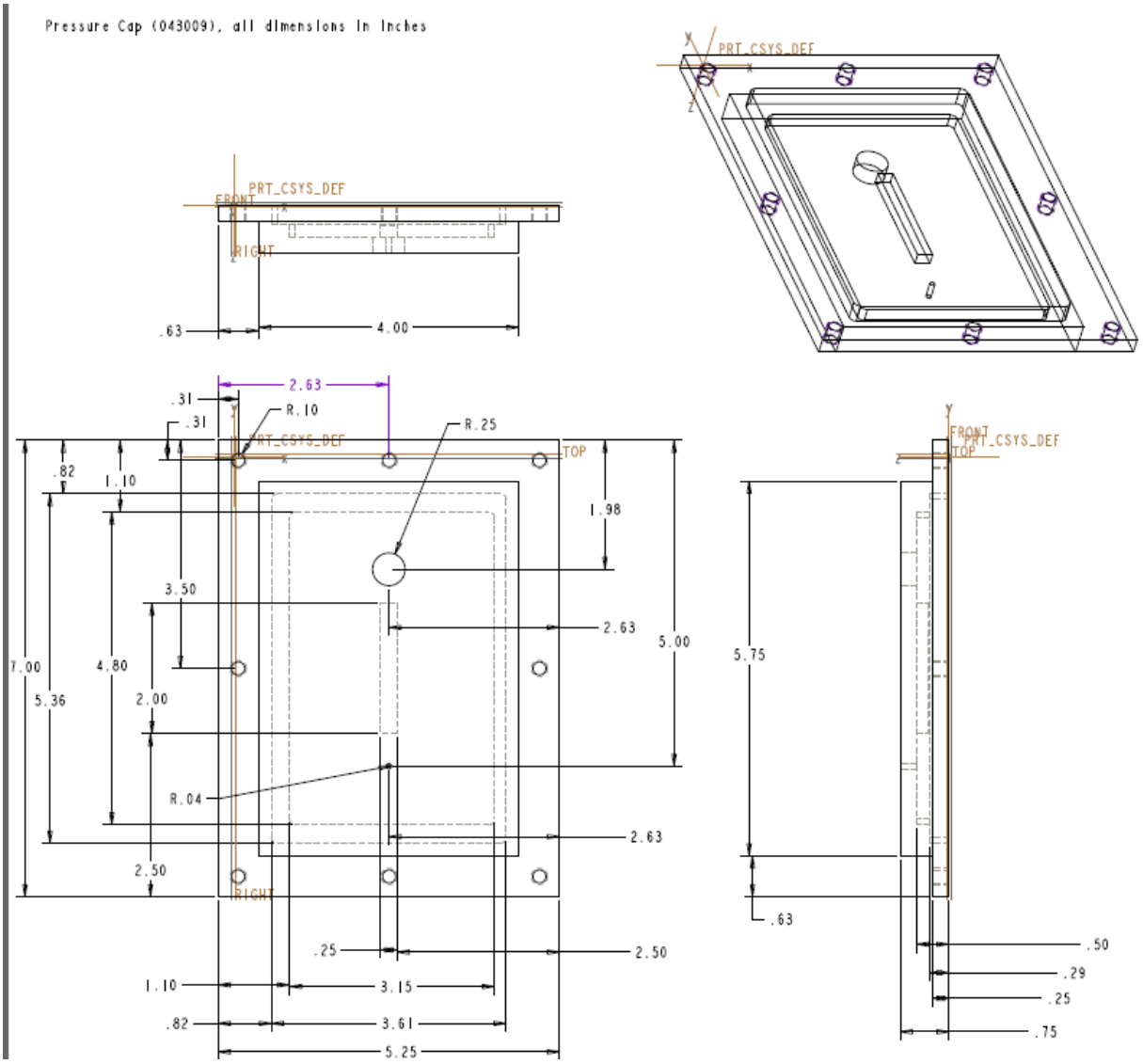
Appendix D

	Categories	Ease of Setup	Number of Parts Involved	Compatibility with Janus Robot	Safety of Operator	Risk of Harm to Sample	Time for Filtration	Risk to Membrane		
Transport Mechanism	Weights	10	5	10	10	15	15	15	Total Score	Ranking
Diffusion	Scoring	8	9	6	8	9	2	8	550	1
Pressure	Scoring	4	5	5	7	7	7	4	455	2
Electroosmosis	Scoring	6	6	5	4	2	9	6	435	3
Electrophoresis	Scoring	6	6	5	4	2	8	6	420	4

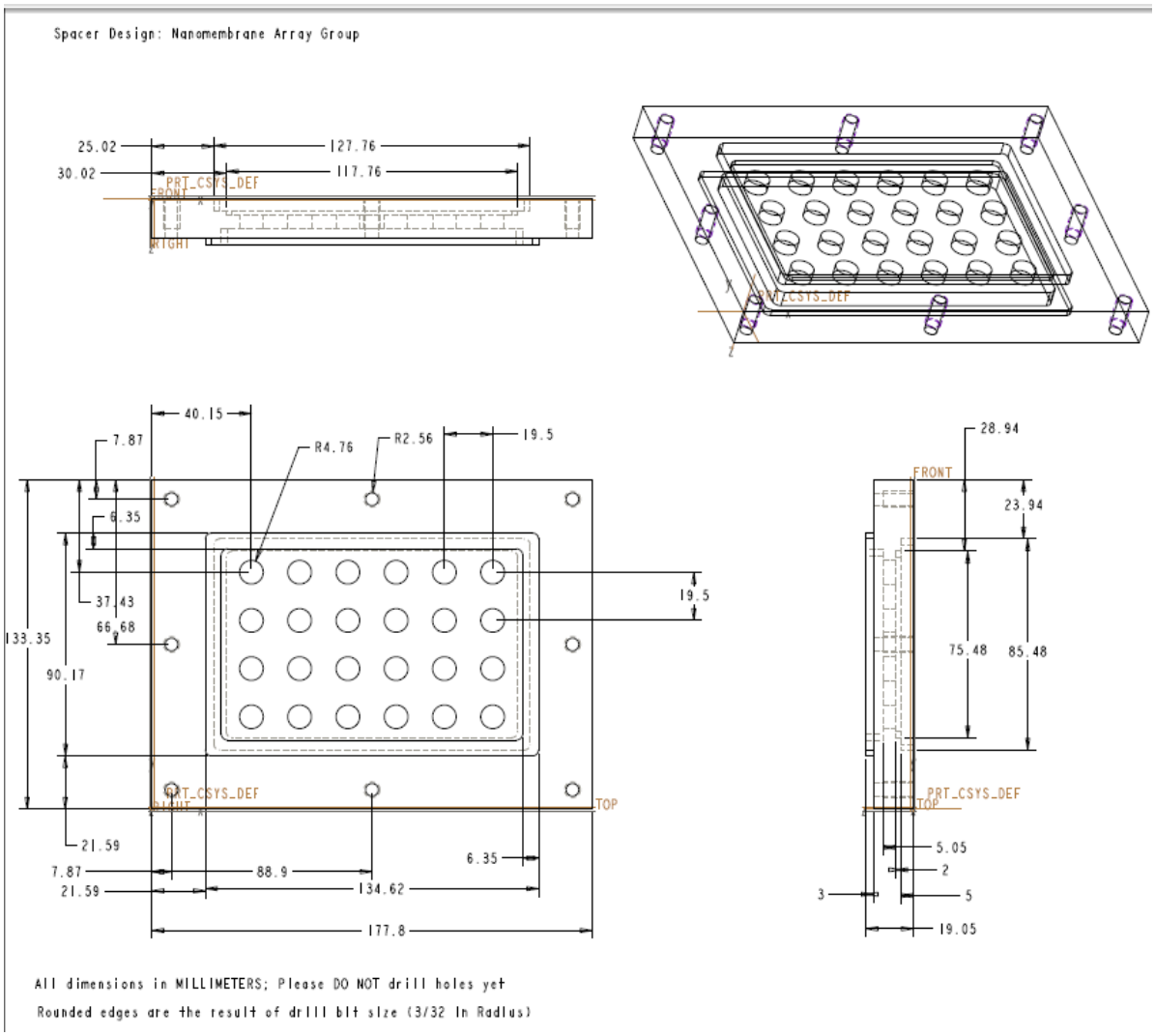
Diffusion, despite performing well in the concept scoring matrix shown, was ruled out because diffusion time would potentially lead to sample denaturing and would certainly decrease our ability to make a high-throughput device.

Appendix E

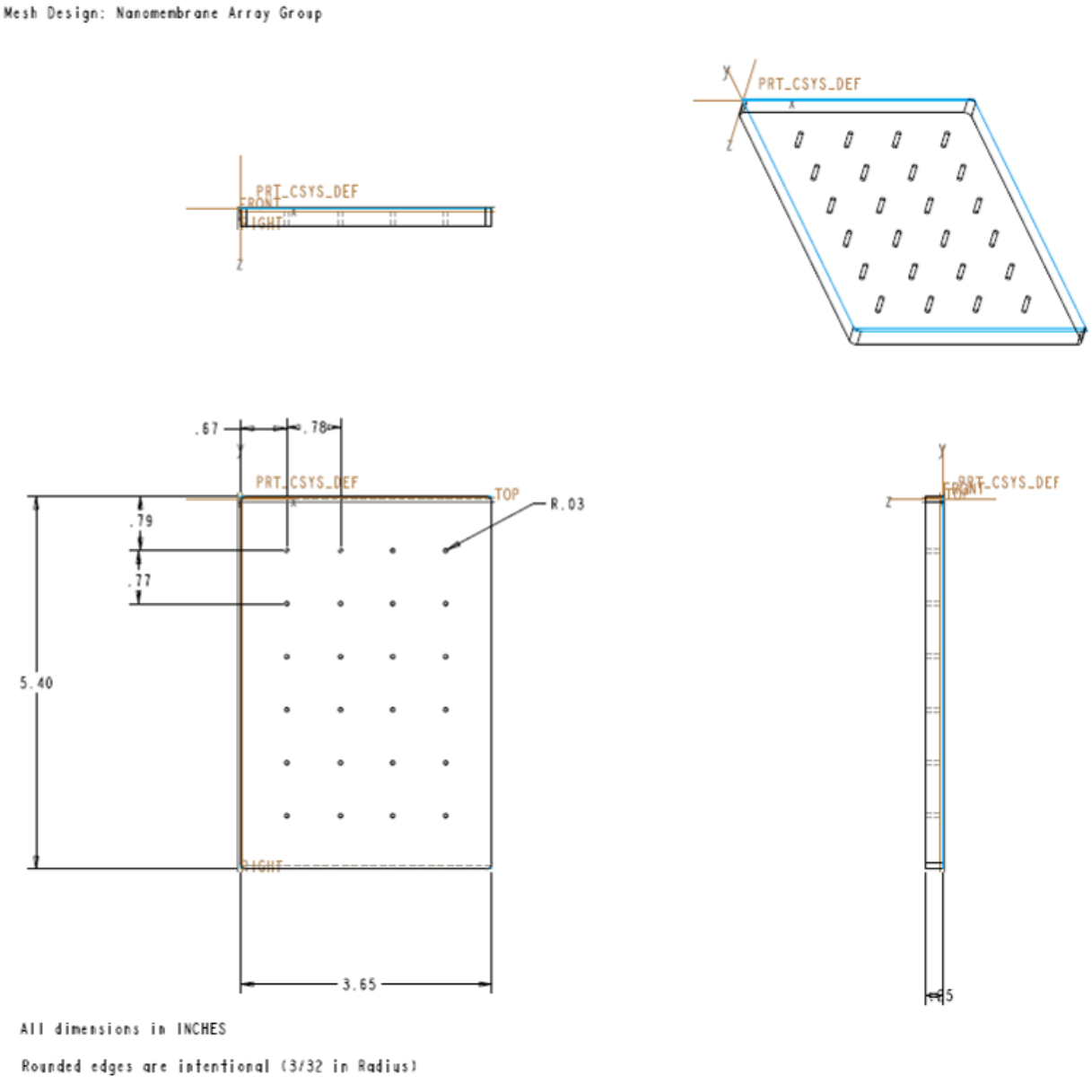
Pressure Cap Component Mechanical Drawing:



Spacer Component Mechanical Drawing:

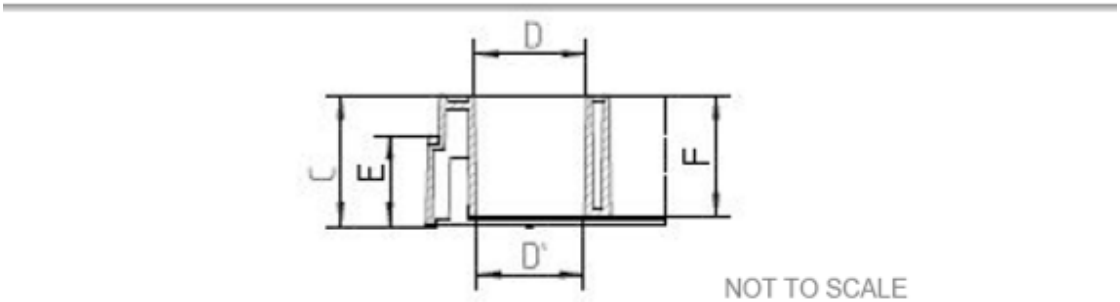


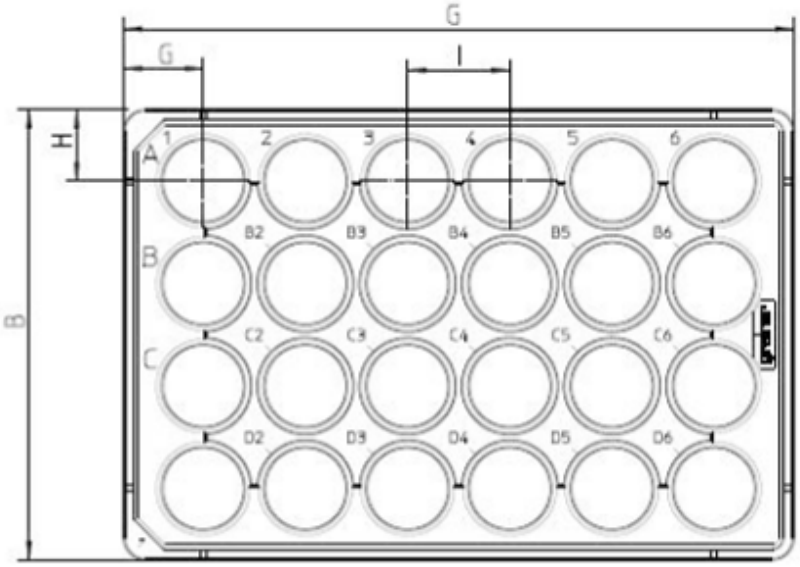
Mesh Component Mechanical Drawing:



Appendix F

24-well Plate Dimensions Sheet:





Cat.-No.	Description	A	B	C	D	D`	E	F	G	H	I
662 892	SensoPlate™	127.76	85.48	19.0	Ø 16.28	Ø 15.56	13	17.7	15.13	13.49	19.5
662 896	SensoPlate™	127.76	85.48	19.0	Ø 16.28	Ø 15.56	13	17.7	15.13	13.49	19.5

All dimensions in mm

Customer drawing subject to change without notice!

Prior Issue	Drawn	Approved	Released	
Revision 01	Date August 30, 2007	Date August 30, 2007	Date August 31, 2007	CONFIDENTIAL: Information contained in this document or drawing is confidential and proprietary to Greiner Bio-One GmbH. This document may not be reproduced for any reason without written permission from Greiner Bio-One GmbH. All rights of design, invention, and copyright are reserved.
Date November 9, 2005	Name F. Falter	Name F. Sonntag	Name F. Sonntag	

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