The Design of an Ideal Substrate for the Reproducible Cell Morphology and Nitric Oxide Production of Endothelial Cells

Team 08 - Proposal

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Executive Summary

Measuring nitric oxide (NO) production from endothelial cells in vitro is difficult due to the molecule having a very short half life. NO production is dependent upon proper cell morphology because proper elongation determines the placement of caveolae, the site for production of NO by endothelial nitric oxide synthase (eNOS). Endothelial cells elongate to form an aspect ratio of approximately 2.0, similar to in vivo endothelial cell elongation, when they are grown on a grooved substrate with the dimensions of 3.5 μm groove width, 3.5 μm spacing width, and 1 μm groove depth. Our objective is to reproduce these dimensions on a porous membrane so that NO diffusion across the membrane can be measured. A micro-groove design must be transferred onto the surface of a porous membrane by means of an accurate and affordable manufacturing method. This method should allow for grooved membranes to be reproducible and have little interference with diffusion across the porous membrane. The final design will yield a guided, porous membrane that can be used for testing endothelial cell function and development. Although the design is intended for experimental use, if successful, the patterned porous membranes developed could potentially be useful for high throughput testing.

This report details the current state of the prototype which includes the photomask and its duplication, silicon wafers used to fabricate PDMS substrate test layers, and copper substrates used for electroplating. These are all crucial steps in reaching our final goal of producing macro-grooved porous membranes. The photomask, which is used during the photolithography process to produce substrate molds for PDMS layers, is very expensive and fragile. This is why the mask duplication is an important process to ensure that prevent any type of extensive damage to the original photomask. These duplicate masks consist of glass substrates with a layer of chrome on the surface. A layer of photoresist is placed on the surface of the “chrome mask” and is clamped
together with the original mask. By exposing the masks to UV light, the exact micro-pattern on
the original mask is duplicated on the chrome mask. The state of the silicon wafers we have
created are also detailed in the report. The silicon wafers are used as molds used in the soft
lithography of the PDMS test layers. This is achieved by spinning photoresist onto the wafer,
baking it to harden the photoresist, and exposing it to UV light through the photomask. The
wafers are then submerged in a developing solution which removes the exposed regions to
produce the desired micropattern. Several of the trials performed creating these silicon wafers are
detailed in the report including samples of both trials that produced damaged and successful
results. Copper plating is also an important step in our design process, since these substrates will
be used to transfer the desired micropattern onto the porous membranes during hot embossing.
This is achieved by creating a Ni pattern on a copper substrate through an electrochemical
process.

Problems and Issues that the team has encountered throughout the design process,
such as ambitious scheduling issues are discussed. Issues that are expected to be encountered,
such as those with hot embossing, are discussed as well. The team’s plan of action for the spring
term is provided in detail leading up to the final presentation. The societal and environmental
issues, albeit small, are detailed as well and project to be mostly positive.
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I. Introduction

Measuring nitric oxide (NO) production by endothelial cells lining blood vessels is useful for circulatory research. Endothelial Nitric Oxide Synthase (eNOS) produces NO when present on the cell membrane of endothelial cells at sites known as caveolae. In vivo endothelial cells elongate in response to flow in the blood vessels and contain caveolae on the surface exposed to flow. Endothelial cells grown in vitro on a PDMS substrate without exposure to flow are round with no elongation and also contain eNOS centrally located in the cell. Exposure to flow causes cell elongation and eNOS to be present on the surface of the cell membrane in the caveolae.

Similarly, cells grown on a PDMS substrate with a micro-groove pattern exhibited cell elongation and NO production from the surface caveolae. Successful growth of endothelial cells is achieved when cells cultured in vitro exhibit the aspect ratio of in vivo cells. The aspect ratio (the length of the cell in the direction of flow divided by the width) of in vivo endothelial cells is greater than 2.0. Dimensions of 3.5 μm groove width, 3.5 μm spacing width, and 1 μm groove depth are expected to produce the desired aspect ratio (Barbee et al., 1994). This will produce an extensive fibrous network formed along the longitudinal ridges, characteristic of endothelial cells under shear stress, which simulates cyclic loading experienced by cells in vivo.

NO production is difficult to measure due to the molecule’s short half-life. When using PDMS substrates a NO-sensing electrode is placed inside a flow chamber to measure NO productions. The concentration gradient within the flow chamber causes a steep variation in concentration near the wall of the chamber, causing the concentration measurements made by the electrode to be inconsistent. Incorporating micro-grooves onto the surface topography of a
porous membrane enables endothelial cells to elongate and subsequent NO production to be measured through diffusion across the porous substrate.

II. Solution

A micro-groove design must be transferred onto the surface of a porous membrane by means of an accurate and available manufacturing method. This method should allow for grooved membranes to be reproducible and have little interference with diffusion across the porous membrane.

III. Design Constraints

The selection of Polyethylene Terephthalate (PET) as the porous substrate will be a constraint for this design. PET was chosen as the membrane material due to success in previous work, and for the fact that it is a biocompatible material that facilitates efficient cell attachment. Polyester (PET) porous membrane transwell inserts manufactured by Corning, Inc. will serve as the membrane onto which the desired micro-pattern will be transferred. The manufacturing specifications and properties of the transwell membranes include a membrane diameter of 24 mm, a growth surface area of 4.67 mm², a pore size of 0.4 µm or 3.0 µm (2 possible manufacturing models), and a nominal pore density of 4 x 10⁶ pores/cm² (0.4 µm pore size) or 2 x 10⁶ pores/cm² (3 µm). The micro-groove pattern on the porous substrate will be 3.5 µm groove width, 3.5 µm groove spacing, and 1 µm groove depth in accordance with preliminary data.
Hot embossing will be used to transfer the pattern onto the PET membrane. Hot embossing works by heating PET just above its glass transition temperature ($T_g$) and then applying pressure. This manufacturing method was selected because hot embossing equipment is available, while other manufacturing methods which may have improved resolution, such as laser etching, are not available.

IV. Design Criteria

Our solution is two-fold: first, to grow endothelial cells on a grooved substrate with proper morphology (measured by the aspect ratio), to ensure the presence of caveolae and thus NO production, and second, to reproduce the grooved pattern on a porous membrane.

Cell morphology will be measured using the aspect ratio. The aspect ratio of in vivo endothelial cells is greater than 2.0 with an average width of 10 μm (Barbee et al., 1994). Preliminary data has shown cells with their axes aligned within +/- 20% of the channel direction exhibit proper elongation and caveolae distribution. When various groove widths and depths were tested, maximum alignment (90% of cells) occurred in groove with 3.5x 3.5x 1μm spacing on a PDMS membrane (Uttayarat et al., 2005). It is expected that with these dimensions 90% of the cells will exhibit proper elongation which grown on the PET substrate.

A photomask containing the 3.5x 3.5x 1μm groove design was replicated using photoresist and ultraviolet light exposure. Following microfabrication, which includes methods of soft lithography and hot embossing, the groove pattern will be replicated from the original photoresist onto porous PET. The walls of the grooves on the PET substrate need to be as vertical as possible with an acceptable error of ±°25. Assuming a constant volume for the compressed substrate, the maximum applied pressure can be calculated for the elastic modulus of
the substrate. The force applied cannot be so great as to close the pores. To determine the exact
pressure to be applied, a numerical simulation will be conducted using software such as ANSYS.
The simulation will be used to analyze the stress distribution.

The hot embossing of the micro-grooved pattern onto PET is expected to result in partial pore closure. This procedure will require the construction of a cylindrical fixture attachment which will align the PET membrane with Si wafer pattern during hot embossing. The temperature must be between the PET glass transition temperature \( T_g \) of 80 °C (176 °F) and a melting temperature \( T_m \) of 254.4 °C (490 °F). At the correct temperature, PET will not lose its viscosity, which causes the PET to flow sideward. The sideward flow will result in the nano pores on the porous membrane to close (Wooley et al., 1993).

After applying pressure, the system is allowed to cool, and the mold is then separated from the PET membrane. The hot embossing process is expected to decrease the pore area. Unmodified membranes with a pore diameter of 0.4 microns have been successfully used. The pore area fraction of those membranes is 0.5% of the membrane volume. Membranes with pore area fractions less than 0.5% are deemed unsuccessful.

Nitric oxide diffusion across the porous membrane can be measured using a NO-sensitive electrode. Ideally, both elements of our design will be incorporated so that the flux of NO produced by endothelial cells across a porous membrane can be measured. The flux can be predicted using a simple linear diffusion model. Two experimentally determined values of the diffusion coefficient \( D \) of NO at 37°C and 25°C have been measured by a porphyrinic sensor using linear scan voltammetry and found to be \( 3.30 \times 10^{-5} \text{ cm}^2\text{s}^{-1} \) and \( 2.60 \times 10^{-5} \text{ cm}^2\text{s}^{-1} \), respectively. Using Fick’s first law of diffusion, the diffusion process can be derived as:

\[
C = C_0 \text{erf} \left( \frac{x}{2 \sqrt{D t}} \right)
\]
In this equation, $C_0$ is the initial concentration, $C$ is the concentration at a distance $x$, time is $t$, $x$ is the distance from the plane where $c = c_0$ (which will be the membrane’s thickness), and $D$ is the diffusion coefficient which has been experimentally determined as described above. From this equation the expected concentration $c$ can be estimated on the membrane’s surface for a selected time. Any pore closure resulting from hot embossing is not expected to compromise the diffusion of NO across the PET membrane due to the small size of the molecule and the substrate’s high pore density.

V. Description of the Prototype to Date

Intended Prototype

The goal is to fabricate micro-scale grooves on the porous membrane transwell inserts without stopping NO diffusion. The primary solution is to use hot embossing to transfer the pattern on to the porous membranes. The secondary solution is to either print protein patterns on to the porous membranes or use laser micromachining to create the microgrooves on the porous membrane. We need to create a micro-scaled mold on an appropriate substrate that will be used in the hot embossing process as a stamp to transfer the pattern on to the porous membrane and fabricate our prototype. The pattern to be transferred will be decided after cell culturing results show which pattern gives the best NO production/ aspect ratio for the cells from the range of patterns we selected. The mold will be created using a combination of photolithography and electroplating which requires various tools and equipment. For cell culturing molds we would
only need photolithography and softlithography techniques to create substrates that will be used to culture endothelial cells. The figure below shows the AFM image of a silicon substrate with microgrooves. Our goal is to replicate similar kind of microgrooves on a porous membrane. The pattern dimensions of our arrays are different from the ones shown below.

![AFM image of Microgrooves](image.png)

**Figure 1: AFM image of Microgrooves**

**Prototype Fabrication**

The first step in fabricating the prototype is the photolithography process. The various techniques involved in this process allow us to fabricate microgrooves made of photoresist on a polycrystalline silicon wafer. The microgrooves are then transferred onto a PDMS substrate using softlithography. The PDMS substrate can then be utilized to culture cells. The cell culture on PDMS substrates will allow us to choose the pattern to be transferred, which will be the pattern that provides best NO production/aspect ratio for the cells; from the range of patterns we selected.

For the hot embossing process we need to create a mold that will act as a stamp to transfer the desired pattern on to the porous membrane. This mold cannot have a silicon wafer as
the substrate because silicon is very brittle and not an ideal material for the hot embossing process. The hot embossing mold will be made of Copper substrates and Nickel patterns. To fabricate this mold we would need to use photolithography and electroplating techniques. At first we would fabricate our microgrooves on Copper substrates using photolithography. The microgrooves on the Copper substrate will act as an insulating barrier during the electroplating process. During the electroplating process the copper substrate with the photoresist grooves is placed in an electrolytic solution. The applied voltage forces the Ni ions to move towards the Cu substrate, where the photoresist blocks the Ni ions to be deposited onto the Cu substrate. The areas where the photoresist is absent the Ni ions are deposited onto the Cu substrates. After the entire process of depositing Ni is completed, the photoresist is removed using specific developer chemicals and various other techniques. The finished substrate will be used as the stamp in the hot embossing process. To transfer these microgrooves onto the porous membrane we need to raise the temperature of the polyester (porous membrane material) to its glass transition temperature. After this, the Cu substrate is pressed against the porous membrane with the correct amount of pressure to transfer the patterns onto the surface. It is important not to raise the temperature too far above the transition temperature in which case the viscosity of the polyester material decreases too much for the patterns to be transferred effectively. High pressure would certainly damage the porous membrane and low pressure would not allow the polyester to transform enough for the patterns to be transferred. The correct pressure and temperature values for hot embossing will be calculated from stress simulations using software such as ANSYS.
Fig 1: Electroplating

**Current state of the Prototype**

The photomask necessary for the photolithography process was designed by our team using AutoCAD 2009 software and the file was sent to Image-Tec Corporation for manufacturing. We have received the photomask from Image-Tec Corporation and have begun the photolithography process. The first step in the process is to duplicate the photomask from Image-Tec to a chrome mask.

**Mask duplication:** The Original photomask is expensive and fragile. To prevent damage to it from extensive usage we first duplicated the original photomask. The duplicate photomask is used in the photolithography process to create the microstructures. To duplicate the micro-
pattern on the photomask we used glass substrates with a layer of chrome on one surface. Above the chrome layer is a layer of AZ 1500 photoresist. This duplicated mask will now on be referred as the “chrome mask”. The original mask and the chrome mask are brought into contact and held together by using mechanical clamps. The patterned side of the original photomask and the photoresist side of the chrome mask should face each other. The two masks are then placed under a UV light source with the original mask facing towards the UV light source. The photoresist layer is exposed by UV light in specific areas where the photomask does not block the UV rays. After the exposure there is a change in the physical and chemical properties of the photoresist. The substrate is then placed in an AZ 450 developer solution which removes the exposed photoresist. The substrate is then placed in a chrome etchant solution. The solution removes chrome from areas where the photoresist has been removed. After this step the exact micro-pattern on the original photomask is duplicated on to the chrome mask.

Fig 2: Duplication process
The initial try of the chrome mask copying resulted in a residue formation on top of the substrate. This was probably due to dust particles and PR residue on the substrate. The mask was then cleaned in a solution of piranha to dissolve residue. Piranha, also called Piranha etch, is a solution of concentrated sulfuric acid (H\textsubscript{2}SO\textsubscript{4}) and hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), with a ratio of 3:1 (3 H\textsubscript{2}SO\textsubscript{4} : 1 H\textsubscript{2}O\textsubscript{2}). It is highly acidic and is a common chemical to be used for cleaning in microfabrication processes. The dust particles were dislodged by placing the mask in a water bath in the sonicator. The mask was further cleaned using fiber-free swabs to remove any remaining residue. The pictures below show the mask before and after the cleaning process, these pictures were taken at the Bio-MEMS lab by our team:

![Before cleaning](image1.png)  ![After cleaning](image2.png)

**Fig 3: Piranha cleaning of chrome mask**

**Silicon wafer:** We had previously failed to make a successful mask on a silicon wafer due to expired photoresist. The new photoresist was received during week 5. Since then we fabricated several substrates with different fabrication steps.

The mold fabrication process includes the following steps in their respective orders:
- Buffered oxide etching (BOE): removes the thin layer of silicon oxide on the surface of the silicon wafer. This step improves the adhesion of photoresist to the Si wafer.

- HMDS primer application: the primer again improves the adhesion of the photoresist to the silicon wafer.

- Photoresist spin coat: In this step we apply photoresist onto the Si wafer and place it in a spin coater, which spins it at various speeds for a desired amount of time. The faster the spin speed the thinner the layer of Photoresist (PR).

- Soft bake: This step hardens the PR so that it does not stick to the chrome mask during exposure.

- Exposure: In this step we expose the Si wafer for a specific amount of time. This step transforms the chemical and physical properties of the exposed region, rendering it soluble in the photoresist developer.

- Developing: The exposed wafer is submerged in a developer solution which dissolves the exposed region.

- Post bake: This step further hardens the PR so that it can be used for softlithography.

- The following table shows how the parameters were changed to make different molds:

<table>
<thead>
<tr>
<th>Try</th>
<th>BOE</th>
<th>Primer</th>
<th>Spin speed (RPM)</th>
<th>Spin time (s)</th>
<th>Exposure (mJ/cm²)</th>
<th>Developer solution (DI water:developer)</th>
<th>Developing time (s)</th>
<th>Agitation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>no</td>
<td>1000</td>
<td>25</td>
<td>192.8</td>
<td>250:100</td>
<td>30</td>
<td>yes</td>
</tr>
<tr>
<td>2</td>
<td>yes</td>
<td>no</td>
<td>1200</td>
<td>30</td>
<td>192.8</td>
<td>300:100</td>
<td>30</td>
<td>yes</td>
</tr>
</tbody>
</table>
All the tries showed varying results. The various pictures of the microgrooves were taken under the microscope and are displayed below:

The substrates with no BOE etching showed the worst results. The substrates with no primer coating also showed very bad results.

From the 4 trials till now we can assume that BOE etching and primer coating is vital for a good groove formation. The higher spin speed also resulted in a different type of damage, which was probably caused by poor contact between the substrate and the mask.

The pictures below show the difference between a good microgrooves and damaged ones, these pictures were taken at the Bio-MEMS lab by our team:

Good Fabrication:

Damaged Array Patterns:

Table 1

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>no</td>
<td>yes</td>
<td>1200</td>
<td>30</td>
<td>192.8</td>
<td>250:100</td>
</tr>
<tr>
<td>4</td>
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<td>1200</td>
<td>30</td>
<td>192.8</td>
<td>250:100</td>
</tr>
<tr>
<td>5</td>
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<td>yes</td>
<td>1400</td>
<td>30</td>
<td>192.8</td>
<td>270:100</td>
</tr>
</tbody>
</table>
A: Damage due to agitation,

B: Damage when no BOE was performed and no primer was applied

C: Damage due to poor contact

From the results of our fabrication trials till now we can see that BOE and primer application are necessary for a good mold formation. We also have to optimize our spin speed to get the correct thickness of our PR layer, a good thickness will result in better contact between substrate and mask.

The type of pattern damage was specific to their respective cases, namely excessive agitation, absence of BOE and absence of adhesion primer, i.e. the type of damage when agitation was performed was different from the type of damage when no BOE was carried out.

From the various steps taken away we were able to determine that the PR adhesion to Si surface and contact between the substrate and photomask are the two major problems in fabrication of the array patterns. The PR adhesion was improved by carrying out both BOE and application of HMDS primer solution. To improve the contact between the substrate and the photomask we had
to make our PR layer more uniform. To do this we carried out two spin coats instead of one at higher RPM. The higher RPM ensures that the PR thickness is more uniform; however the thickness of the PR layer decreases. To increase the height of the PR layer we carry out multiple spin coats. After every spin coat the substrate is soft baked, this hardens the PR layer and increases its viscosity, so that in the second spin coat the first layer acts like a substrate and does not lose its thickness. The new photolithography parameters (BOE and HMDS primer application steps remain the same as before) are stated below:

- BOE and HMDS primer
- Spin coat: 1st coat: 3000RPM - 1000RPM/s - 30s
- Soft bake: 2min @ 100ºC
- Spin coat: 2nd coat: 3000RPM-100RPM/s - 30s
- Soft bake: 8mins @ 100 ºC
- Exposure: 192.8 mJ/cm2
- Developing: 30s no agitation. (270ml : 100ml - water : developer)
- Hard bake for 8min at 100 ºC

From this process we were able to get excellent results. We had all patterns well developed with only one exception. Few patterns showed very minimal damage but only in small areas of the array. During cell culture the array patterns with minimal damage will provide a large enough area for cell attachment. From this we were able to conclude that 2 spin coats showed better results than a single coat. The images of some of the array patterns are shown below.

![Multiple spin coat Silicon wafer](image)
Copper substrate: The photolithography process for the copper substrate requires a negative photoresist (PR) for fabrication. The exposed part of the PR remains on the substrates acting as an insulator for Ni deposition on Cu. This allows us to get the same Ni pattern on the Cu substrate as the one on the photomask. For this process we used SU-8 2002 photoresist.

Figure 5: Copper substrate fabrication

To remove the Photoresist after electroplating we heat the copper substrate to 150ºC and immediately put it into a cold water bath. Due to the difference in the coefficients of thermal expansion between copper and PR, the PR experiences large tensile and fractural stresses. This cracks the PR and allows it to dissolve in Acetone much more easily. Acetone is used to remove the PR only and not the Ni.

The intended height of our pattern is 5µm on the porous membrane, same as the height of the Ni deposit. The height of the photoresist on the copper substrate however should be approximately
2-4µm higher than the intended height of the deposited nickel. This is to ensure that the Ni being deposited on the substrate does not grow over the PR thickness. This will result in the Ni to start growing laterally rather than horizontally.

![Figure 6: Ni thickness greater than the PR thickness](image)

The thickness of our PR for 5µm patterns should be approximately 7-9µm. The maximum resist thickness of SU-8 2002 is 3µm in one spin coat. To get our intended height we carried out multiple spin coats. We were only able to carry out the photolithography part due to time constraints. The photolithography steps are shown below:

- Su-8 2002: 1ml Pr for every 25mm or 1 inch of wafer diameter
- Spin coat: 1st coat: 1000RPM - 200RPM/s - 30s
- Soft bake: 2min @ 95 ºC
- Spin coat: 2nd coat: 1000RPM-200RPM/s - 30s
- Soft bake: 3mins @ 95 ºC
- Spin coat: 3rd coat: 1000RPM-200RPM/s - 30s
- Soft bake: 4mins @ 95 ºC
- Exposure: 165 x (1.7) = 280.5 mJ/cm²
- Post Exposure Baking (PEB): 4mins @ 95 ºC
- Develop: 40min - extreme agitation
- no hard bake.

All the array patterns on the Cu substrate were not completely developed. We were able to obtain 5 good array patterns from the fabrication. The surface of the Cu substrate was not polished enough. There were also various contaminants observed under the microscope. The image of one of the patterns is displayed below:
The copper substrates need to be cleaned with Piranha to remove any contaminants. A more polished substrate will also show much better results. Our team is working on eliminating these problems from the fabrication process. We are confident that our team will be able to fabricate a good copper substrate for electroplating soon.

VI. Problems and Issues

There have been a few issues that the team has tackled so far and may run into throughout the life of the project. The first stage of our design project required the design of a photomask to be used in the micro-fabrication process. This photomask design was sent out to an external company, Imagtec, and required about a full month of the fall term to be constructed before we could begin working with it. Although a lag time was naturally expected and factored into the scheduling of the project, it did limit the amount of preliminary data which we could
obtain. It was originally planned to have already optimized the micro-pattern by the time fabricating substrate molds would begin. This has set us behind schedule from where we would like to have been at this point with the project. It is hoped that we will be able to make up some of the lost time during the initial micro-fabrication process.

Another issue regarding the design project is problems with of hot embossing. Several problems are expected to be encountered when working to use hot embossing on the porous membranes. Optimizing the process in terms of time, temperature, and pressure is going to be a huge factor in determining the success of the design. Also the design of a suitable apparatus to be used in conjunction with the hot embossing process to properly facilitate the use of the hot embossing press on the fragile trans-well inserts, will be another factor in determining success. This apparatus must allow for proper transfer of the micro-pattern to the porous membrane insert, but not cause a significant alteration to the membrane’s physical properties. It may turn out that the available equipment in the MEM lab may not be suitable to creating patterns this low on the micron scale, since our specifications are of considerably smaller resolution that previously attempted here at Drexel University. The team has consulted several studies showing that hot embossing on this small scale is indeed possible, and has proposed several possible novel ideas that could aid us in the hot embossing process. Of course, if hot embossing is ultimately ineffective, new methods of approach will be attempted such as laser ablation or micro-contact protein printing to mimic the substrate dimensions.

VII. Plan of Action for Spring Term

Currently PDMS test substrates and corresponding substrate molds are in the process of being fabricated. This, along with cell culture being conducted on each PDMS test substrate in
order to confirm ideal dimensions of the micro-pattern to produce appropriate cell morphology, will enable the finalization of a hot embossing protocol and hot embossing assisting apparatus to be completed by week 10 of the winter term. A desired micro-groove pattern with the dimensions of 3.5 μm groove width, 3.5 μm spacing width, and 1 μm groove depth has been identified as our target substrate topography, and data collection will be finalized in the coming days leading up to hot embossing trials.

This sets the ground work for what will be done during the spring term. Attempts to hot emboss the micro-pattern onto the PET porous membranes will begin during week 10 of winter term, upon finalization of the copper-plated substrates. Confirmation of hot embossing success will be measured by analyzing the fabricated porous membranes with microscopy such as, SEM or AFM, in order to analyze porosity and integrity of the membrane after hot embossing, as well as the substrate topography dimensions. This will be done once the porous membranes have been fabricated with hot embossing, most likely in the beginning in the middle of March. Assuring cell adhesion to the fabricated PET membranes after hot embossing is possible will be the next step. If hot embossing turns out to be not possible with the currently available equipment, the first weeks of the spring term will be devoted to designing a laser ablation protocol, followed by cell culture of those membranes fabricated by this method. If either method is successful, microscopy will be used to verify cell attachment was successful no later than the 3rd week of the spring term. If problems also arise with the laser ablation process, then one last approach will be taken. This approach involves protein printing, in which thin lines of adhesive proteins are printed directly onto the substrate of the porous membrane through micro-contact printing, which mimics the desired micro-pattern. Since this is somewhat of a last resort, this method would most likely take place fairly close to the end of the design process leading up to the final report in
about week 7 of spring term. The remaining portion of the spring term will be spent preparing
the final project report (due around May 11th), business report, and final presentation.

VIII. Societal and Environmental Impact

This micro-patterned porous membrane design, which allows for reproducible endothelial
cell morphology, and a means to measure nitric oxide production, has several implications in the
field of biomedical research. The substrate design will aid in efficient and reproducible testing
involving endothelial cells and the construction of blood vessels in tissue engineering
applications. Potentially, the design will enable researchers to more effectively study endothelial
cells, their morphology, and their production of nitric oxide. Successful implementation of this
design could reduce the need for animal models when researching endothelial cells.
Pharmaceutical agents could also be tested in a small scale setting as opposed to an animal study.

This design is intended for experimental testing, thus the scope does not entail mass
production, and thus only small quantities of chemical and material waste will be generated.
Some chemicals, especially those used to develop the photoresist, are toxic and carcinogenic.
The Material Safety Data Sheet (MSDS) of each chemical will be consulted and proper
laboratory practices will be followed when handling them. The environmental impact of our
design will be minimal because the hazardous byproducts will be produced on such a small scale,
and will be properly disposed.
### IX. Schedule Including Items Completed

<table>
<thead>
<tr>
<th>Tasks</th>
<th>Start Date</th>
<th>Duration</th>
<th>End Date</th>
<th>Completed</th>
</tr>
</thead>
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<td>9/10/2009</td>
<td>21</td>
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<td>✓</td>
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<tr>
<td>Decided upon microfabrication techniques with Dr. Noh and Dr. Barbee</td>
<td>10/20/2009</td>
<td>11</td>
<td>10/31/2009</td>
<td>✓</td>
</tr>
<tr>
<td>Researched similar devices and their fabrication</td>
<td>10/10/2009</td>
<td>60</td>
<td>12/29/2009</td>
<td>✓</td>
</tr>
<tr>
<td>Created Photomask using AutoCAD</td>
<td>10/20/2009</td>
<td>30</td>
<td>11/19/2009</td>
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<td>Hot embossing transfers micro-pattern onto PET polyester pore membrane</td>
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<td>Endothelial cells cultured on PET polyester porous membrane</td>
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<td>Cell morphology measured using microscopy imaging software</td>
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<td>NO release measured through porous membrane in flow chamber</td>
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X. Gantt Chart Detailing Project Timeline

References:


Appendix:
Table of Possible PET membranes

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<tr>
<th>Membrane Type</th>
<th>Pore Density</th>
<th>Pore Diameter</th>
<th>Pore Volume Fraction</th>
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<tr>
<td>A</td>
<td>4.0E+10</td>
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<td>C</td>
<td>2.0E+10</td>
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Pore density is measured in pores/m^2

Pore Diameter is measured in μm.

The three types of membranes have a diameter of 0.012 m and a thickness of 10 μm.