

Lung Tissue Chamber For the Study of Asthma

Group Members:

Tom Neils
Eric Miller
Kevin Kinney
Megan Toth
Rafael Connemara
Andrea Schnelle
Barry Bass
Adam Graf

Advisor:

Mark Nicosia

Clients:

Dr. Julie Kessel
Dr. Julie Sedgwick

Date:

12/14/01

Abstract

The incidence of allergy and asthma is an ever-increasing health problem in our world today. A great deal of research is currently being directed toward determining the cause of these diseases. Lung tissue research at the cellular level deals with three individual cells; the endothelial cells lining the blood vessels in the lung, the epithelial cells lining the air sacs of the lung and the white blood cells that migrate from the blood to the lung. These cells are isolated and grown in culture so that they can later be analyzed using microscopy, molecular biology and tests of cell function. If these cells could be grown together in a system and environment that is similar to an actual functioning lung, the relationship between them and how they react to each other in an allergenic environment could be more greatly understood. This system needed for this experiment will provide an adequate way of testing the cells asthmatic response to antigens/allergens. There are no current devices that incorporate endothelial cells, epithelial cells and life-like flow.

The final design is a three piece device with a base that allows for growing of the epithelial cells in the correct orientation, a space for a fluid matrix in between the two cell layers, and a piece that allows for flow over the top of the endothelial cells mimicking blood flow. The three pieces are then clamped together to insure a proper seal, thus creating a small scale working model of the lung.

Design Problem

The lung is a complex organ where the exchange of oxygen and carbon dioxide occurs. In people with asthma there is a reaction of cells to certain allergens (ie. ragweed) that causes a toxic response. The current method to test this is called a lavage. It is an uncomfortable experience where tubing is inserted down the throat, and allergens are sprayed directly on the lung tissue. The asthmatic will have a reaction to the allergens. This reaction is then quickly suctioned back up by the tubing and then analyzed. This test needs to be improved so it is not at all painful and more accurate.

The design problem is to come up with a device to mimic the lung in a way that it can duplicate this toxic reaction so it is possible to study how and why it occurs. Therefore, this testing device will have to include a layer of endothelial cells, which is the cell layer lining the blood vessels in your lung, and a layer of epithelial cells, which are the cells lining the air sacs of the lung. These two cell layers must be separate by a distance of no more than 5mm and be able to contain a fluid matrix in between them that allows for the migration of white blood cells or components of the blood from one cell layer to the other.

All of this test must be recordable by videomicroscopy, and be able to run for up to 72 hours. Then once the test is complete, the cell layers and the fluid matrix between them must be accessible for further analysis. Then, if possible the components of the design should be able to withstand sterilization and then be reusable.

Background Information

Asthma is debilitating both physically and emotionally for people around the world afflicted with the disease. The number of asthma cases worldwide is on the rise. This disease is a result of the lung having an exaggerated immune response and hyper-

inflammation induced by various antagonists. Some of these antagonists include allergens, pollutants and exercise. Some examples of airborne allergens are ragweed, pollen, dust mites, and animal dander.

Current research focuses on three cells found in the lung tissue; the endothelial cells lining the blood vessels, the epithelial cells lining the air sacs, and white blood cells that migrate in from the blood to the lung in response to the presence of an antagonist. The specific type of white blood cell that causes the exaggerated immune response in asthmatics seems to be eosinophils. These eosinophils are a major force in the immune system. Their main function is to combat parasites or foreign cells. Within the lungs of those suffering from asthma and other lung diseases the number of eosinophils covering the endothelial tissue is much higher than for normal individuals. In addition, it has been found that the amount of eosinophils in the lung tissue greatly increases during an asthma attack. According to Dr. Julie Sedgwick, “if you stop the incoming eosinophils, you could stop the toxic response.” This would be a major focus of the tests that would be run with the device we are working on developing.

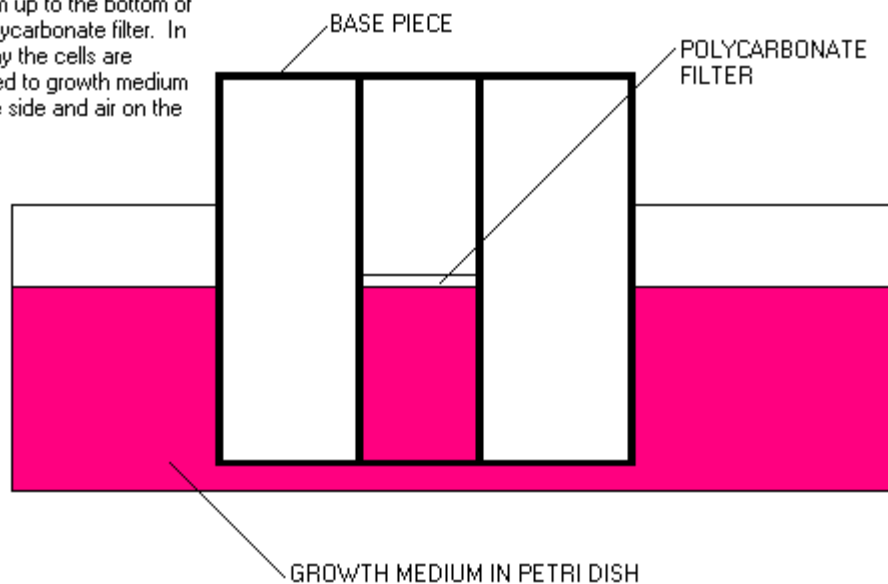
The epithelial and endothelial cells function in unique ways. The endothelial cells have gaps and tight junctions, which allow specific white blood cells to migrate through them and attack foreign materials, bacteria or parasites. The epithelial cells lining the inside of the lung have cilia, which are small hair-like projections that slow up invading antigens allowing the immune system to attack the invaders. In all allergenic responses these two cell types work together to recruit the necessary white blood cells.

As soon as allergens come into contact with the epithelial cells they are recognized and the immune response begins by histamines being released. The histamines, among other things, cause the endothelium to secrete a protein that promotes eosinophils to attach to the problem area as they pass into the blood stream. The affect these cells have on the endothelium and epithelium is very important to allergy research. Scientists feel that they will be able to investigate the correlation between asthma and the higher levels of eosinophils.

Design Constraints

There are several problems that were important to address in our design of the asthma-testing device. First of all the cells in the lung are very well organized and have a

FIG. #1 The base piece submerged in growth medium up to the bottom of the polycarbonate filter. In this way the cells are exposed to growth medium on one side and air on the other.



specific orientation. The epithelial cell layer is the first exchange interface to encounter the air we breathe into our lungs. These cells have small hair-like cilia that are exposed to the air. Therefore, when the cells are grown for use in the asthma testing they must have the same orientation as in the lung. Our base piece that contains the epithelial cells must allow for this proper growth when the cells are being incubated. We solved this problem by attaching the polycarbonate filter, on which the cells are grown, into a hole of the base piece that leaves the filter exposed on the top and the bottom. This way the piece with the filter in it can be submerged in a growth medium up to the bottom side of the filter and the top of the filter can be exposed to the air to allow for correct orientation of the cells (Fig 1).

Another problem was ensuring a seal when the test blood is flowing over the endothelial cell layer. This was overcome by using rubber o-ring material around the areas exposed to the test blood and then clamping all of the components together to keep a tight fit in between pieces. This also keeps all of the pieces from moving or shifting during the experiment that could last up to 72 hours.

For further design constraints see PDS in appendix A.

Alternative Solutions

One solution to the problem of creating a small-scale model of the human lung would involve sliding and locking two slides into the flow chamber. Each slide would have a polycarbonate filter that allows growth of the appropriate cells. For example one slide would have a filter with endothelial cells grown on it and the other slide would have epithelial cells on it. These individual slides, once in the flow chamber, would be separated by a space filled with the interstitial fluid.

The slides used for this test would have to be specialized with a hole in the middle that is filled with the polycarbonate filter. Possibly two slides with holes could be stuck together, and the filter is placed in between.

FIG. #2 Specialized Slide
The slide necessary for this experiment would have to have a hole in it that is spanned by a polycarbonate filter. This filter is the growth substrate for the desired cells.

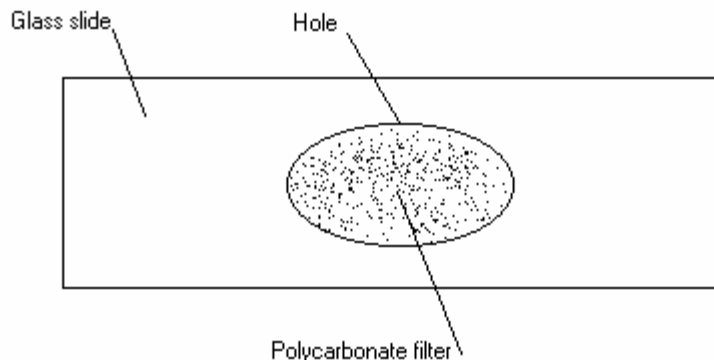
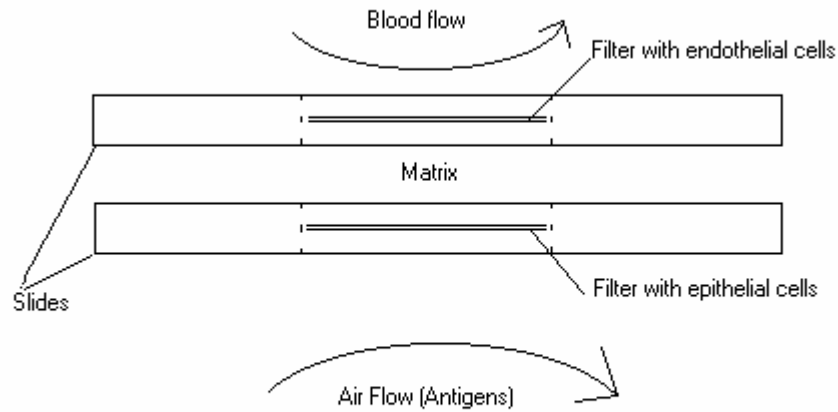


FIG. #3 Side view of slides in flow chamber



The best way to set up this test would be to have the slide containing the epithelial cells placed in the flow chamber. These cells would have to be orientated downward with the cells exposed to the air. The matrix could be filled in on top of the first slide then the other specialized slide containing the endothelial cells would be placed on top of the matrix. This would have to be sealed so that the blood could flow over the top.

Another solution to preparing this model would be to have a one-piece cassette that is inserted into the flow chamber. The two types of cells would be grown separately on polycarbonate filters then each piece that the cells are grown on would be put together. This would have to be sealed so a fluid matrix could be injected in between.

FIG. #4 Cassette with matrix being injected.



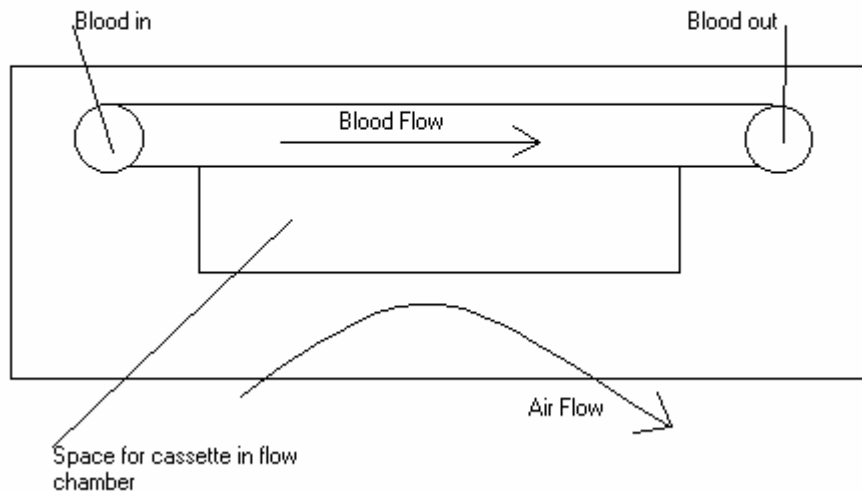
FIG. #5 Cassettes



With this method the cassette would be able to be split in half the long way, and each half would contain a polycarbonate filter that the desired cells would be grown on. The cells would be grown separately on each half of the cassette then the two halves

would be sealed back together and the matrix would be injected in through a septum on the side. Once the two halves are sealed back together with the matrix injected in the middle the cassette could be slid into a specialized flow chamber with the epithelial cells orientated downward exposed to the air. The endothelial cells grown on the other half would be exposed only to the flow of blood in the flow chamber (FIG. # 6).

FIG. #6 Side view of flow chamber with slot for cassette



This method of testing the lung cells seems to have many advantages over some of the other designs. The cassette would allow for easy growth of the cells and an effective way to control the size of the fluid matrix in between by simply creating the desired volume of space in between the two cell layers. Once the test is over the matrix could be removed from the cassette using a syringe and the cassette could be disposed of.

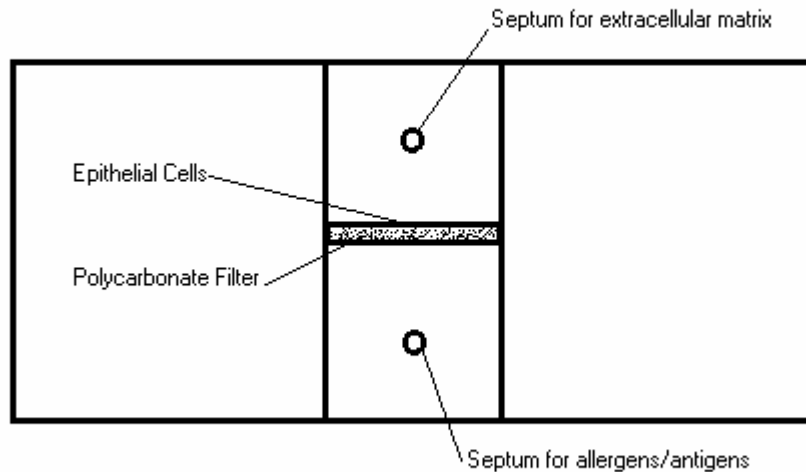
A problem that could arise using this device is that the filter used to grow the cells on is very delicate. If the area created for the cells is too large it could be too flimsy and easily damaged. This must be taken into consideration when handling the cassette and prepare the cell cultures. Also, with this design the space containing the matrix will have to be sealed so that none of the interstitial fluid can leak out. This would not be a problem if we did not have to grow each half of the cells separately, but since the two pieces of the cassette must be resealed it could be tough to make it water tight. Once the cassette is placed in the flow chamber it must have a tight seal where it comes in contact with the blood flow. This may also be a challenge since the cassette would be removable.

Due to the complexity of in diagrams a third alternative design may be viewed in Appendix B.

Final Design

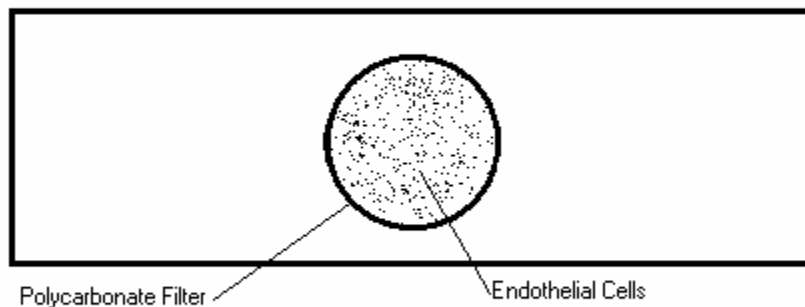
The design that we chose to build for this project was a simple model, but it adequately fulfilled all of the requirements. It has 3 main components that are clamped together to hold them tight and create a seal in between the pieces that contain test blood flow. The base piece is a block of clear polycarbonate with two sizes of holes drilled in. In the middle of this block rests a polycarbonate filter that supports the epithelial cells. The bottom hole allows for the allergen/antigen to be introduced and the top hole allows for the fluid matrix, that lies in between the two cell layers, to be introduced (FIG. #7).

FIG. #7 Side view of base piece for final design.



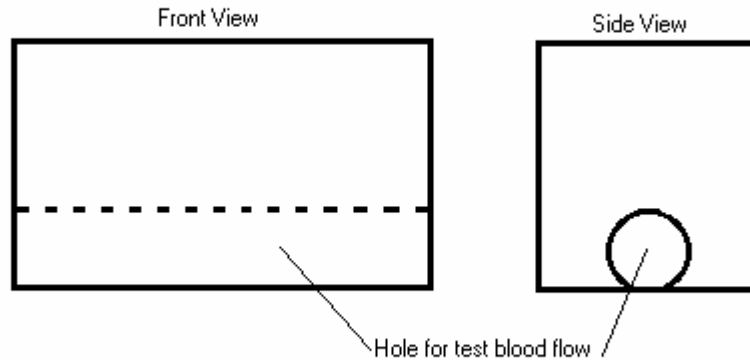
The second piece to the design is a thin slide-like piece of polycarbonate. It also has a hole drilled in it that lines up with the hole in the base piece. The hole in the slide is also spanned by a polycarbonate filter that has the endothelial cells grown on it. This piece will have the test blood flow over it (FIG. #8).

FIG. #8 Top view of slide piece that contains a hole with a polycarbonate filter that has the endothelial cells grown on it. This piece had the test blood flow over it.



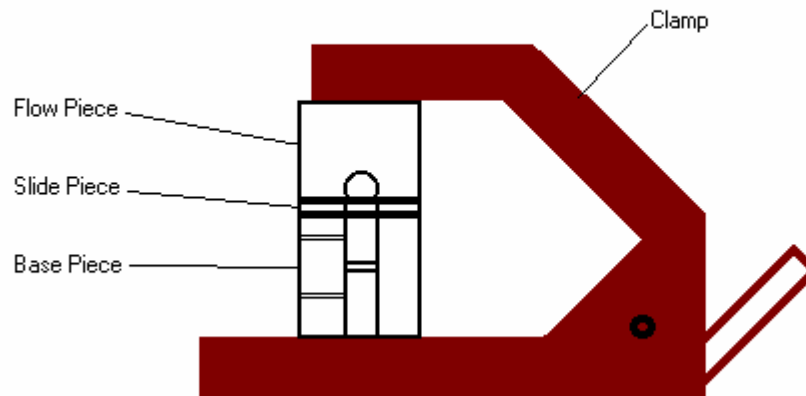
The third component to our design is the top piece that allows for flow of the test blood over the endothelial cells. This piece has a hole that passes through it which is connected to tubing that is in turn connected to a pump. This piece is quite solid and strong because it will be the top piece where the clamp pushes down (FIG. #9).

FIG. #9 Flow piece with hole passing through it allowing for the test blood to pass through it over the endothelial cells.



All of the components will be clamped together with the base piece on the bottom, the slide in the middle, and the flow piece on the top. These will be tightly forced together to keep a seal when the test blood is flowing over the endothelial cells (FIG. #10). All of these pieces together will complete the model of the lung, with epithelial cells being introduced to an allergen/antigen, then their response is sent through the fluid matrix and received by the endothelial cells. The endothelial cells then react with the cells in the test blood. This reaction is what scientists desire to observe and analyze.

FIG. #10 The clamp hold all three of the components together creating a seal between the Flow piece and Slide.



Conclusion

Asthma is one of the most common obstructive airway diseases today. This fact provides the need for a device to better understand the complications in the human lung during an asthma attack. Current research is restricted to one part of the reaction that causes the asthma attack due to the fact that the device that is used for testing does not include both the endothelial and epithelial cells. We believe that we have designed a product that will now accurately represent the entire actions of the human lung. Ideally, with this design, the proper research for asthma will be completed and a greater understanding will follow.

We believe that our final design is a strong step in the direction towards providing an accurate model for testing asthma, but there are several areas to work on before the final product could be produced. Some of these areas include; repeated testing to ensure durability, proper mechanics, and accuracy. As with any preliminary design, the testing will probably point out flaws in the design that would need to be corrected. With successful testing we would hope that our product could be used for wide spread asthma testing. If our model of the lung is successful, the concept could easily be altered so that it could be applied to other areas of research. Our client suggested that with slight modifications this design could be used to study tissues in the bowels and placenta.

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Appendix A: Tissue Culture Chamber-PDS

12-14-01

Team Members:

Tom Neils(leader), Adam Graf(communications), Eric Miller(BWIG), Kevin Kinney, Barry Bass, Megan Toth, Rafael Connemara, Andrea Schnelle

Function:

The function of our product is to mimic the gas exchange within the alveolar sacs in the lung. If one can view this exchange, the results may be very helpful for the study of asthma. Our device will give an exact copy of this procedure, which includes the following:

- Constant blood flow over endothelial cells (blood vessels)
- Accessible interstitial fluid (matrix-space between)
- Correctly orientated epithelial cells (inside alveoli, air)
- Direct contact of air, allergens etc. to epithelial cells

Client Requirements:

- The device must allow for epithelial cell growth in the correct orientation.
- The device must allow for blood flow over endothelial cells.
- The device must contain an airtight seal around epithelial cells
- The device must be made of materials that are currently being used in testing procedures.
- **The matrix/interstitial fluid should consist of a minimum of .1 to .5 ml or 100 to 200 micro liters min.**
- **The most important feature—the matrix must be able to be accessed otherwise unable to view exactly what goes on during a asthmatic/allergic reaction**

1. Physical and Operational Characteristics:

a) Performance Requirements:

- The device must be able to withstand repeated use for non-disposable components.
- The device must be able to withstand repeated handling by technician as well as varying blood flow.

b) Safety:

- The device should be sterile and easy to clean.

c) Accuracy and Reliability:

- The device should be easy for technicians to correctly fill with matrix fluid and manipulate.

d) Life of Service:

- The device should be durable enough to last through the period of the growth of the lung tissue (~3 weeks) , and also the time required to perform analysis of the system(~24-48 hours).

e) Shelf Life:

- The device should have a long enough shelf life to be stored on the shelves of a laboratory until time of use.

f) Operating Environment:

- Will be used at room temperature. Extreme temperatures may affect experimental results.

g) Size:

- The device's chamber should be no larger than 1 cm³ and filters that are 3-5 μm in pore size.

h) Weight:

- The device should be as light as possible for optimal efficiency.

i) Materials:

- The device should contain filters made of polycarbonate and use polyethylene for the remaining components.

j) Aesthetics, Appearance and Finish:

- The device should be transparent to help aid viewing of tissue and interstitial matrix fluid.

2. Production Characteristics:

a) Quantity:

- Mass product of disposable units.
- One non-disposable blood flow piece that is connected to a blood pump.

b) Target Production Cost:

- As low as possible, depending on varying costs of plastics used, the clamp used, and also the type of the blood pump being used .

3. Miscellaneous:

a) Standards and Specifications:

- While researching the FDA site we found no standards on this type of device.

b) Customer:

- The customer would prefer a device that would make laboratory procedures as easy as possible.

c) Patient-related Concerns:

- Since there is no patients involve with this device, there are no patient related concerns.

d) Competition:

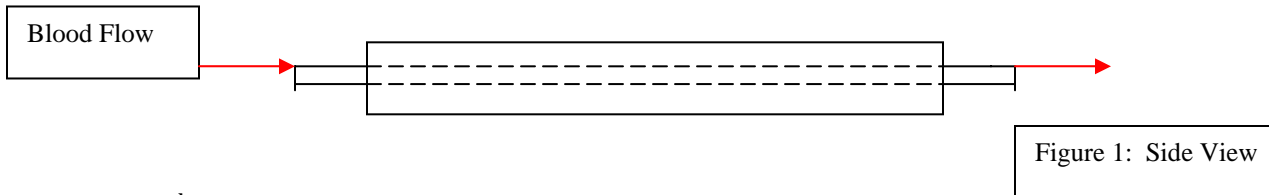
- There are current lung culture chambers, but none that contain a combination of endothelium and epithelium tissue growth, while allowing for blood flow over the complete system.

Appendix B: Alternative Designs

Another alternative design consists of three rectangular boxes that will create the tissue chamber. This design is titled the **Canal Design**.

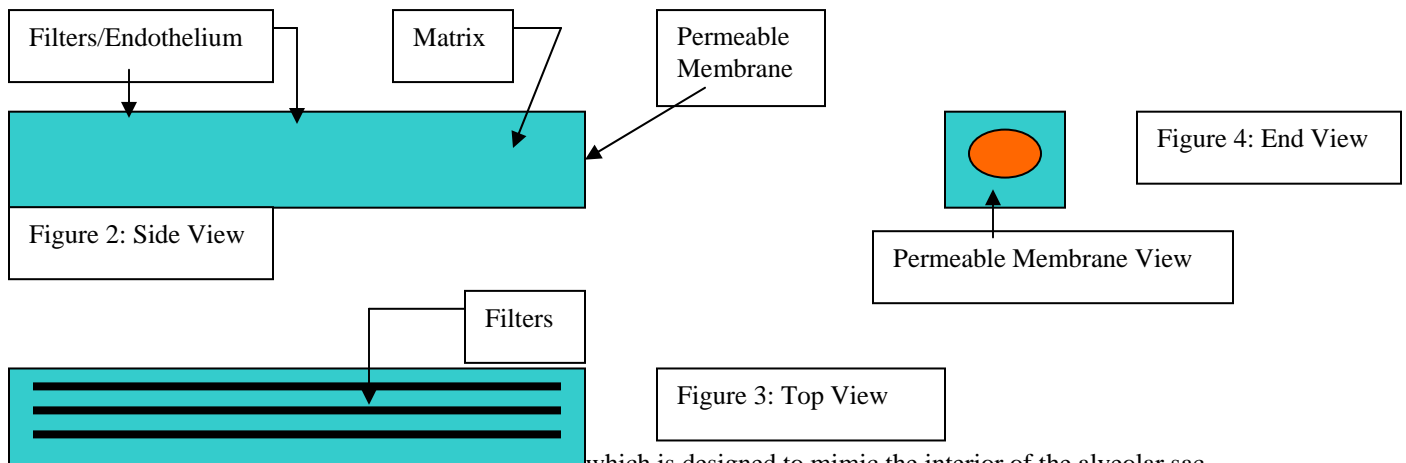
1st Piece:

The first/top piece consists of a tunnel like canal, which will serve as the blood flow over the endothelial cells. The main purpose here is to mimic the blood vessels surrounding the alveolar sac.



2nd Piece:

The next piece, endothelial section, consists of the following: The top is in the shape of a long canal type structure. On the base of this canal structure are a series of filters. These filters are long and narrow. This type of shape will allow the blood flow to have maximum contact area as well as maximum contact time. Directly below this canal, and still attached is another portion of our design, the matrix container. This container is a small area where the matrix will be held. A very important feature of this area of our device is that this liquid inside must be able to be accessed. To do this one side of the container is a membrane, which can be punctured with a needle or syringe to add or sample the fluid inside. The needle/syringe can be removed and the hole left will seal itself. This part is the most important part of the whole device because this is where the sample will be taken, and the sample has all the information, which we would like to study. It is very important that sampling will not disrupt the experiment; that is why we chose this special type of membrane. Samples can be taken and fluid can be introduced without taking the whole device apart. There will be no bottom to this piece because the epithelial piece, the next piece, will need to be directly attached to it.



The last piece is the epithelium piece, which is designed to mimic the interior of the alveolar sac. What this part consists of is a filter, which when set on a level surface will be slightly raised. Around this filter are four walls, which will take the shape of a rectangle. A detachable cover will be the top to these four walls so the epithelial cells will be completely contained except for the septum, which is located in the center of the cover.

This final piece to the device is very unique. The cells, which grow on the filter, need three weeks to grow as well as the presence of matrix. Because of this the bottom filter is slightly raised. The area underneath is for the matrix when growing the epithelial cells. The epithelial device piece is simply put in a dish such as a lab dish where inside the dish a small amount of matrix is added. This way the epithelial cells are able to grow in the right orientation, which will give a correct representation of the whole device. Each of these pieces are fastened together using epoxy. We decided to use epoxy because we felt it was the best way to carry out the experiments. We needed to be sure the matrix area is completely sealed as well as

the blood flow area to provide not only accurate results, but also a safe environment, which the experimenter is able to do his/her research.

