

Introduction

We are designing a tissue culture chamber that mimics a portion of the lung for the study of asthma. This model must include the normal human stresses of blood flow and mechanical forces applied from the airspaces of the lung. It must also support epithelial and endothelial cell layers in manner so that it is accessible for alterations and additions through the air space and/or blood flow. The interstitial fluid matrix must also be accessible, along with a path of sight to the endothelial and epithelial layers after 48 hours (when test is complete). Our design should be generally low in cost with disposable components to allow for many experiments to be performed. This design will be used in the study of asthma to better determine the effects and reactions of specific antigens to the epithelium layer in a more human environment that what is currently available.

Background

Asthma is the most diagnosed illness in hospitals making the study of asthma an extremely important factor in today's society. One important area to concentrate on is the epithelial cell layers that lie on the outer part of the lung air sacs, which are exposed to the air we breath. Next to this layer of epithelium cells is a different layer of endothelial cells separated by a small volume of interstitial fluid. Currently we are not aware of the reactions that occur in this fluid in patients with asthma and allergies. Our client believes that if we know more about this area we will have a better understanding of how asthma and allergies work and may develop better treatments to stop this greatly growing illness. Presently, our client uses a device that only consists of an endothelial cell layer and blood flow. This limited study only partially mimics the lung ignoring a very important factor in what may help us learn more about this illness. By designing a device that better mimics the lung we hope that we may greatly improve the study of this illness.

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 distance that can be 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 (**Fig 1**). Possibly two slides with holes could be stuck together, and the filter is placed in between (**Fig 2**).

Fig. 1 Specialized Slide

The slide necessary for this experiment would have to have a hole in it that is filled with the polycarbonate filter. The filter is the growth substrate of the desired cells

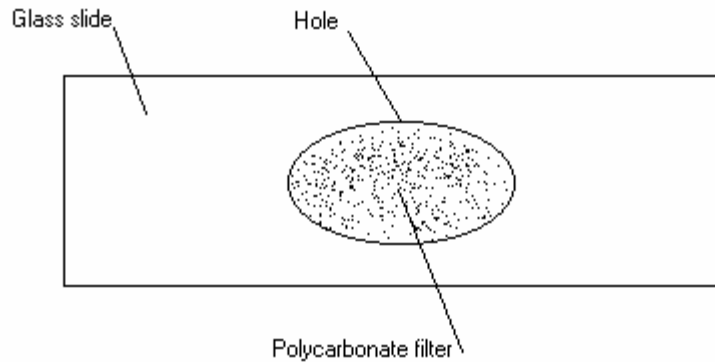
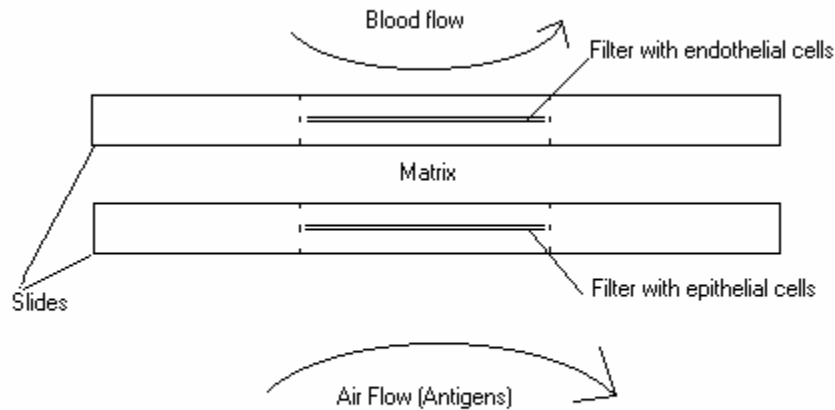


Fig 2 Side view of slides in flow chamber



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 then 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 the blood could flow over the top without leaking.

Unbound Filter

Another preliminary design has a free carbonate filter. Free, meaning that its not attached to any base. It would work as follows. A cylinder would be made of plastic that with a wide base (this base is only used for growing the cells and contain only the fluid used to grow cells) and an accessible tube coming from that wide base (Fig 3.1). This tube is used to change the growing medium regularly as requested by the client. The free filter would be placed on top of the cylinder and pressed down by a cover to keep the filter restrained while cells are grown (Fig 3.2). After these cells are grown onto the polycarbonate filter they can be placed into the simple three-layer stack (Fig 3.3). Because the filter is free we don't have to worry about having the cells grown on the correct face. Here there would be some sort of air tight gel placed in-between layers to keep it airtight. This is a very simple solution to a complex problem. Some cons to this idea are as follows. The matrix size would be a set amount and if the client would feel the need to change this it would require a while new apparatus. The durability of this design is rather limited. If the client would like to move it the lateral movement would go against the gel (the only thing making this air-tight) possibly causing it to slip and loose the matrix fluid. And finally this design is set and therefore is acceptable little if any adaptations.



Fig. 3.1

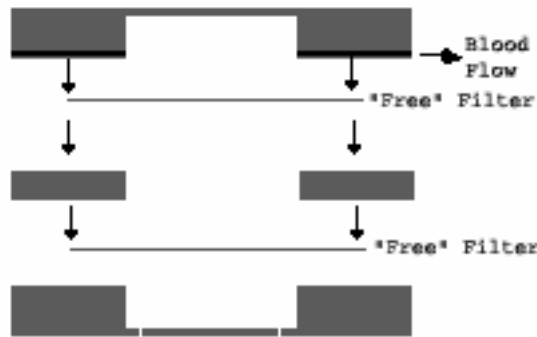


Fig. 3.3

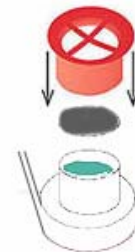


Fig. 3.2

Threaded Cylinder

The last solution that we derived for solving this problem was a design that could be screwed together. This design would enable the user to vary the matrix size according to their experiment. First the base (Fig. 4.1) would be tipped upside down and have the cells grown onto the polycarbonate filter. To do this an apparatus filled with a growing medium would sit flat of a surface. The base with the filter in place would then be screwed onto this apparatus. To change the fluid, one would unscrew the base, which would then allow access to the apparatus core. After the cells are grown the base is allowed to be flipped because of its symmetry. This allows for the cells to be grown in the correct position. Next an apparatus (Fig. 4.2) is screwed into the top (used to be bottom) of the base. Depending on how far the top apparatus is screwed in varies the size of the matrix. Next the matrix would be filled and have a layer of endothelium cells grown onto

a filter placed on top. It would then be inserted into a device that would seal the top layer onto the top apparatus and allow blood flow over the top. A foundation would then be screwed into the bottom that contained a gasket in the middle to allow for future injections of antigens. The main problem with this design is the complexity compared to the others. This would cause for a longer set up time taking time away for the study of interactions.

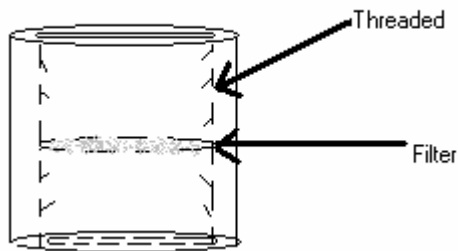


Fig 4.2

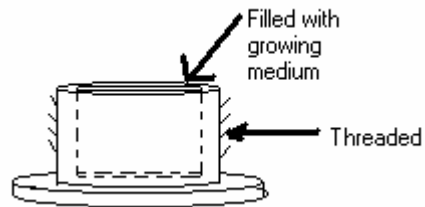


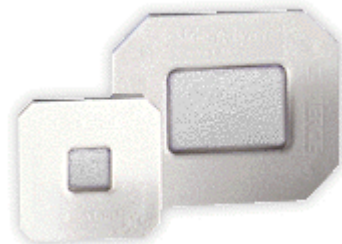
Fig 4.1

Another solution to preparing this model would be to have a cassette that is inserted into the flow chamber (**Fig 3** and **Fig 4**). 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 3**).

Fig 3 Cassette with matrix being injected.
Cassettes



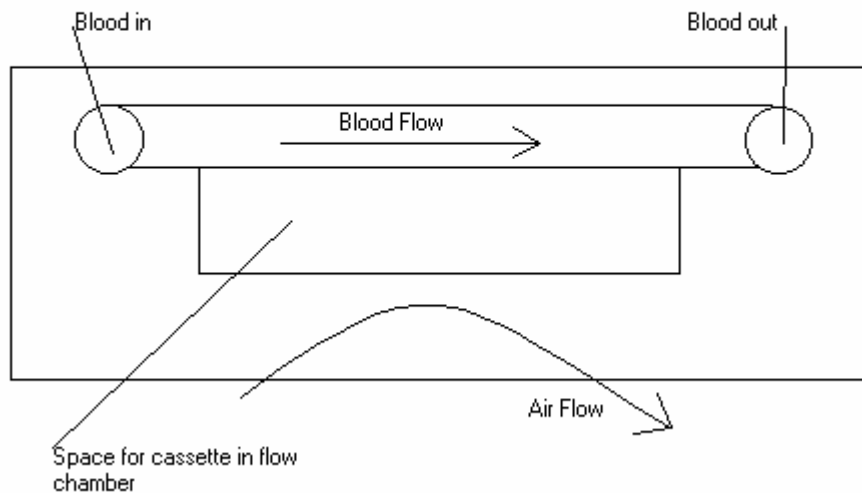
Fig 4



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. So the cells would be grown separately on each half of the cassette than 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 5**).

Fig 5 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.

References

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