

Lung Tissue Culture Chamber

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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.

ProblemStatement

To design a tissue culture chamber that mimics blood flow and mechanical forces in the lung, while maintaining the capability of growing the three main types of lung cells together to be used in the research of asthma and other lung diseases.

Background

Current research focuses on three cells found in the lung tissue; 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. The specific white blood cell type that attack foreign parasites are called eosinophils. Eosinophils are a major force behind the immune system; they combat parasites longer than other white

blood cells. Within those suffering from asthma and other lung diseases the amount 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.

The epithelial and endothelial cells function in different ways. The endothelial cells have gaps and tight junctions, which allow specific white blood cells to migrate through them and attack invading foreign parasites. The epithelial cells within the lung are lined with cilia, a small projection tissue which latches on to invading parasites and allows the white blood cells to attack the invaders. In an allergic response, these two cells work together to recruit the necessary eosinophils. As allergens from the air come into contact with the epithelial tissue histamine is released which, among other things, causes the endothelium to secrete a substance which promotes eosinophil attachment as they pass by in the bloodstream. The effect these cells have on first the endothelium, then the tissue matrix between the epithelium and endothelium, and finally the epithelium is very important in allergy research. By designing a device that mimics the lung in terms of cell position and orientation as well as blood flow and air exposure, the relationship between the effected cells can more easily be viewed. Scientists feel that they will then be able to investigate the correlation between asthma and the higher levels of eosinophils. The following diagram (**Diagram 1**) depicts the relationship between the tissue cells, tissue matrix, and eosinophils.

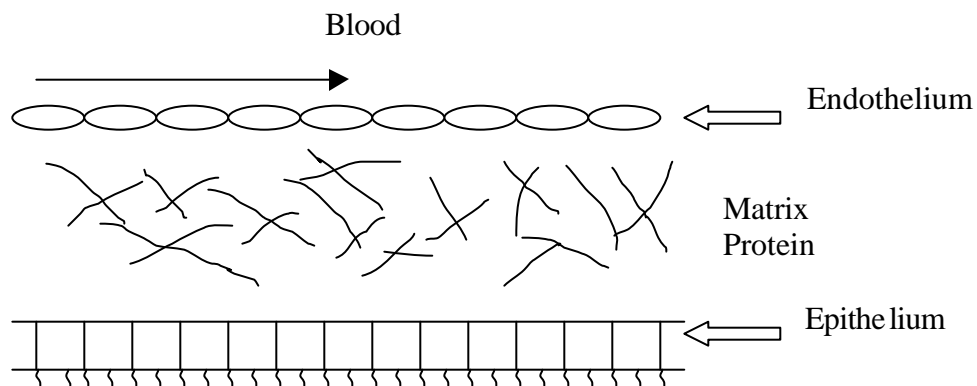


Diagram 1

There are a number of problems currently disrupting the study of the inner lung tissues. First, the epithelial cells must be grown in an upward orientation for correct growth of the cilia. Therefore, they must be grown in an orientation opposite of how they sit in the research mechanism. Another consideration is that of the matrix protein. It must be maintained between the two layers of tissue cells and must allow for researcher manipulation before running the experiment. There must also be a flow of blood over the endothelial cells to mimic the blood flow in a functioning lung and supply the tissue cells with eosinophils. Finally, there must be an enclosed air chamber surrounding the endothelial cells. This chamber should contain a septum for induction of allergens to the endothelial tissue.

Currently, there are only a few methods used in studying the lung tissue. One method used in asthma testing labs here at Madison is the method of growing endothelial tissue on a thin porous plastic filter at the bottom of a small plastic well. This well is then placed into another compartment containing matrix protein. The well is filled with blood containing eosinophils. The migration of eosinophils through the endothelium to the matrix protein can then be studied. This method works but it does not really mimic the

lung because it lacks the second layer of tissues as well as blood flow. A second procedure used throughout the United States is called lavage. A lavage is a procedure where a bronchial tube is run down the throat into the lung and an allergen is pumped into the lungs. After a specified waiting period a saline solution is pumped into the lung to recover reaction material. The material is removed and can later be examined. This procedure can look at the eosinophils after they have reached the epithelium, but lacks the ability to see them at previous stages. In addition, this procedure is very painful and can be dangerous in some situations. Other studies have tried to mimic blood flow over tissue cells (Lawrence), but have found limited success.

Solution

Many elementary ideas came to mind while trying to come up with a feasible solution to this problem. We tried to use some components that were already being used in research procedures. This idea, depicted in diagram 2, allowed for cell growth of both types, and contained area for matrix protein. There were several things wrong with this idea however. First, the blood flow would most likely be turbulent. Second, an effective system of air-tight sealing was hard to devise. Finally, the system itself was not very user friendly and the small components made it easy to damage the fragile tissue cells covering the filter surfaces.

Our proposed solution involves a cassette type device that fits into a fixed system that allows blood flow over one surface of the cassette and air and allergen exposure to the other surface of the cassette. The cassette has a hard plastic exterior for easy handling and an interior space that contains small sheets of the appropriate filter material surrounding a 0.5mL space. The different tissue cells will have the ability to grow on the filter material on either side of the cassette. The 0.5mL space that is found between the filters has the ability to hold the matrix fluid. It is possible to manipulate this fluid if necessary and remove it due to a syringe needle size septum on either end of the cassette. The cassette also contains a channel in it's hard plastic surface. This channel allows for the insertion a fluid compartment that can be used to grow the epithelial cells in the correct orientation. The chamber is simply fit into place on top of the cassette and fluid (nutrients) is added to initiate cell growth on the upper filter surface. The cassette itself can later be placed in a petri dish for endothelial cell growth. When it is time to run the experiment, the nutrients can be removed, the chamber removed, and the cassette placed into the fixed system.

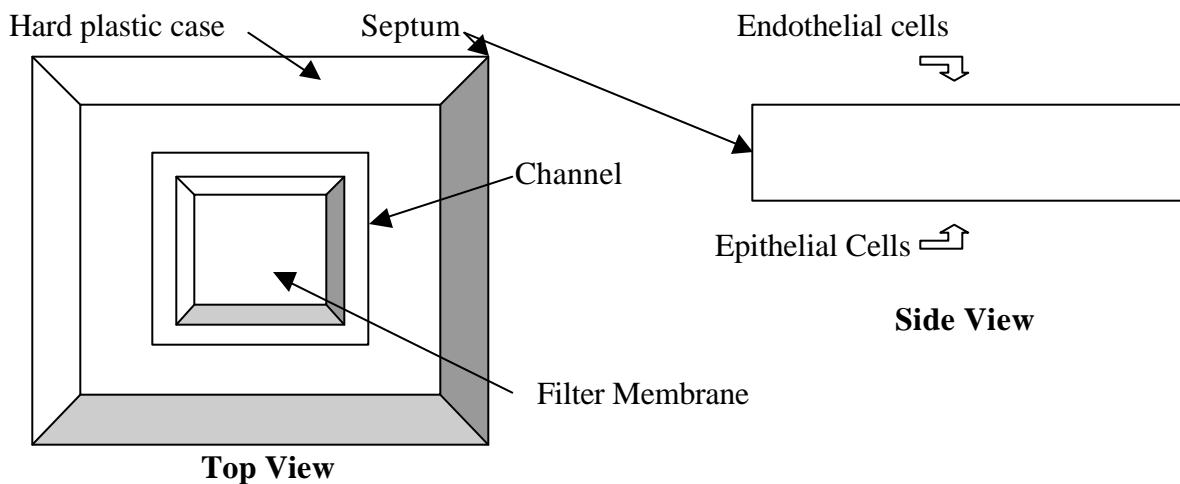


Diagram 2: Cassette

The cassette itself will be placed in a permanent system that contains an air chamber on the side of the epithelium and blood flow on the side of the endothelium. The components are pressed together in a vise type fashion and fitted with o-rings to prevent air or fluid leakage. The blood is pumped through a plastic tube that opens over the area of endothelial exposure. After much research, it seems a peristaltic pump will work best for this application and can be modified to use materials that will work safely with blood. On the opposing side, the air chamber will contain a septum for easy induction of allergens. The cassettes from our device will be disposable. Scientists will also be able to put this device on a microscope for tissue viewing. A few simple drawings of the entire system can be seen in **Appendix A**.

Problems

There are a few problems we may encounter while further perfecting our device. First, getting the blood to flow over the endothelial tissues in the correct manner may be an issue. It is important that all obstacles be eliminated so that turbulent blood flow can be minimized. In addition, we must be able to evacuate the flow tube when the system is taken apart. We must also make sure the cells on each side of the device are secure and not shaken around too much or they may break free from the porous material. Finally, we may want to continue brainstorming ways to grow the epithelial cells in the correct orientation on the cassette, as there may be a better way to do it.

Conclusion

There were many design aspects to consider while doing this project, all of which were accommodated to the best of our ability. Ultimately, the designed system allows endothelium, epithelium, and eosinophils to react together in a system and environment that is similar to an actual functioning lung. Blood flow, air, and the necessary protein matrix are all present. Our configuration allows for the relationship between these cells and how they react to each other in an allergenic environment to be better understood.

Appendix A

