Preliminary Design Report

Perfusion Chamber with Porous Membrane for Cellular-Level Glaucoma Research

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Abstract

Glaucoma is a disease of the eye associated with increased fluid pressure in the ocular chamber that results from a decreased release of fluid through the trabecular meshwork duct. The focus of current research is identification of extracellular matrix peptides that may increase fluid release to reduce the pain and vision loss of glaucoma. The goal of this project is to construct a device that mimics the pressure experienced by *in vivo* trabecular meshwork cells so that peptides may be screened in cellular-level research. The device will control fluid pressure above and below the cell layer and measure the release of fluid with pressure transducers. The final design presented here incorporates a membrane holder and upper and lower pressure chambers sealed to maintain pressure applied to the cell layer. Future work will focus on precise regulation of pressure and ensuring that all device components are compatible with the sterilization technique. Construction of the device will be followed by extensive testing to ensure that the design criteria have been met.

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Problem Statement

The goal of this project is to design and construct a device to control fluid flow across cells of the trabecular meshwork adhered to a silicon membrane. There must be variable pressure from above and below the cells, and the cell culture membrane must be sealed within the pressure device.

Introduction

Overview of Glaucoma

Glaucoma is the 2nd leading cause of blindness in the world. Three million Americans are affected with glaucoma, but only half of them are aware of their condition (Peters, 2007). These statistics highlight the importance of detection and treatment of the disease. Glaucoma is characterized by elevated intraocular pressure, optic nerve damage, and progressive loss of vision (Peters, 2007). Persons with glaucoma experience a loss of contrast and blurriness of vision compared to persons with normal vision (Figure 1).

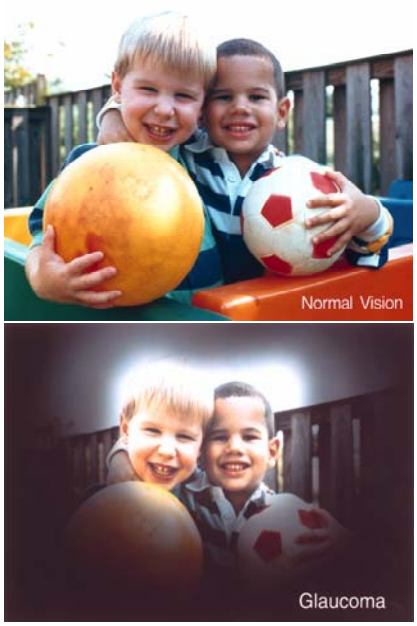


Figure 1. Comparison of normal vision and the vision of persons with glaucoma (National Eye Institute).

The eye constantly produces a watery ocular fluid that fills its anterior cavity (Campbell and Reece, 2005). In the normal eye, fluid drains through both the trabecular meshwork and uveoscleral ducts (Figure 2, Eye Digest, 2007). In eyes affected by glaucoma, these pathways are partially blocked (Figure 2). The result is a buildup of ocular fluid in the anterior cavity of the eyes and an increase in intraocular pressure (Hill *et al.* 2004).

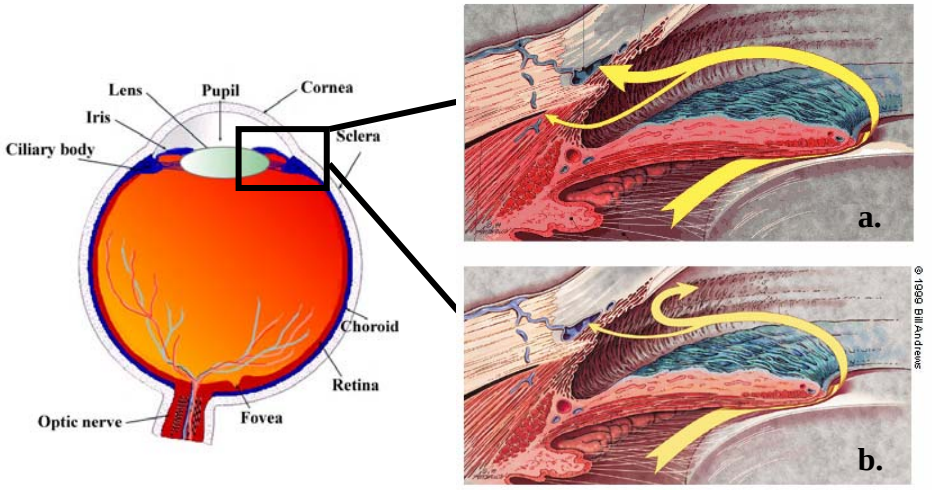


Figure 2. a. Fluid flow in a normal eye through the trabecular meshwork and uveoscleral ducts. b. Blockage of the trabecular meshwork duct caused by glaucoma (Modified from Lewis *et al.*, 1999).

Current Treatment

The main goal of glaucoma treatment is to lower the eye pressure (Figure 3). This can be accomplished by either decreasing the amount of fluid that is produced by the eye or by increasing the amount of fluid that exits the eye through the trabecular and uveoscleral ducts (Eye Digest, 2007). Eye drops are currently the most common treatment for glaucoma, and are intended to decrease the production of ocular fluid. However, eye drops are not ideal. They must be administered one or more times per day, may be difficult to administer directly to the eye, are expensive, and have been shown to cause adverse side effects in some patients (Eye Digest, 2007).

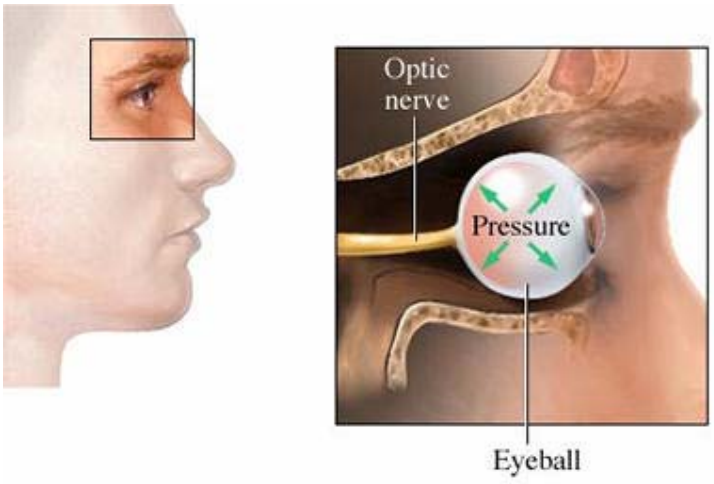


Figure 3. Images of the eye demonstrating the direction of the intraocular pressure (ehealth MD, 2007).

With this in mind our client, Dr. Peters, is working to develop a treatment that can be injected directly into affected eyes that will increase the amount of fluid that exiting via the trabecular and

uveoscleral ducts . A monthly injection would eliminate the difficulty, risk and inconvenience of daily administration of eye drops.

Client Approach and Motivation

Dr. Peters isolates cells from the trabecular meshwork and cultures them on a silicon membrane. She then treats the cells with the extracellular matrix peptide, β-catenin, which signals the breakdown of the actin cytoskeleton of the cells. Fluid pressure is then applied to the cell layer using a Harvard Apparatus® PHD 22/2000 syringe pump to simulate the pressure buildup caused by glaucoma. If the β-catenin effectively signals breakdown of the cytoskeleton, the cells "ball up" and allow fluid outflow. Dr. Peters measures this fluid flow with ISOTEC® pressure transducers that relay information to a computer.

Dr. Peters' overall goal is to identify peptides as potential glaucoma therapies. Initially, she used whole human and pig eyes in experimentation. However, because whole eyes are not readily available, a transition to cellular-level experimentation would save money and allow multiple replicates for faster screening of potential glaucoma treatments.

The current system used by Dr. Peters first creates a fluid pressure from columns of liquid placed above the cell layer, which is supported by an impermeable silicon membrane. This pressure is altered by varying the volume of fluid in each column. This system has several flaws that Dr. Peters would like to avoid in our design. For example, leakage of fluid from the columns interferes with the application of constant pressure to the cells. Additionally, this system applies pressure to only one side of the cells, while *in vivo* cells experience pressure from either side due to fluid of the anterior cavity and a back pressure created by the exterior surface of the eye (Peters, 2007).

Design Specifications

According to the criteria established by our client (Appendix B), the device must be able to withstand a pressure of 40 mm Hg from both above and below the cell layer in order for the experimentation to be as physiologically accurate as possible. It must be compatible with various membranes, so in the future Dr. Peters is not limited to the silicone she currently uses. Dr. Peters plans instead to experiment with more porous membranes, such as hydra-gels. The device must also have the ability to run triplicate experiments simultaneously, and it must be integrated with pre-existing technology that includes ISOTEC® pressure transducers and a computer.

The device will be used daily and must be functional through the current research project, corresponding to a life in service of several years. It must be easily disassembled for UV sterilization and must be constructed of materials that will not corrode with exposure to high-salinity cell culture media. The total cost of device materials must not exceed 3000 dollars.

Design Alternatives

Design One

The primary foci of Design One are allowing incorporation of different membranes into the perfusion chamber and eliminating the problem of fluid leakage experienced with the current device. This design utilizes two plastic rings approximately one-half-inch and five-eighths-inch in diameter. The cell culture membrane is placed over the smaller ring, and the larger ring is placed around the smaller ring and membrane. A small bolt and wing nut tighten the larger ring and seal the membrane in the membrane holder (Figure 4).

The problem of fluid leakage is addressed with a threaded connection between the upper and lower pressure chambers. The chambers are machined from Plexiglas, and the membrane holder rests

inside the lower pressure chamber. An o-ring is placed between the membrane holder and upper chamber and between the membrane holder and lower chamber to seal the system to fluid leakage.

To mimic the internal environment of the eye, a syringe pump introduces fluid to both the upper and lower pressure chambers. The bottom pressure closely matches the pressure expected in the eye of a glaucoma patient, while the upper pressure mimics atmospheric pressure normally experienced at the outer surface of the eye. Pressure outflow from the upper and lower chambers are measured with two pressure transducers. Finally, the individual pressure chambers are supported with wire ring stands for stability during experimentation.

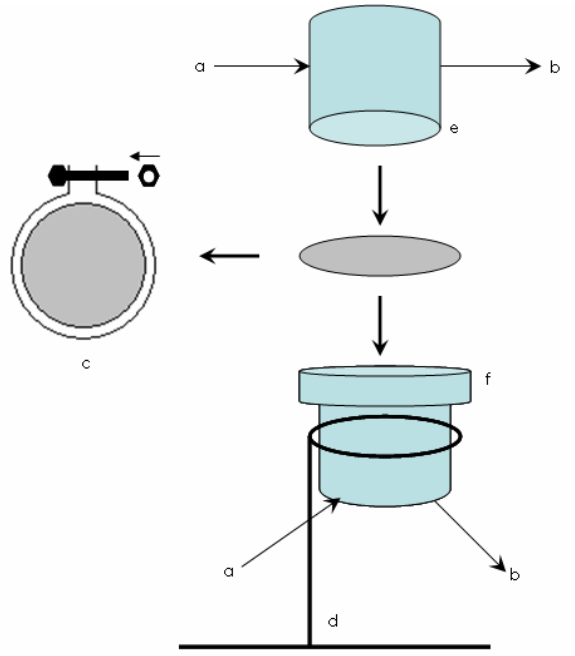


Figure 4. Design one. a. Fluid input from a syringe. b. Pressure measurement at a transducer. c. Membrane holder. d. Device stand. e. Upper pressure chamber. f. Lower pressure chamber.

Advantages

Design one allows for easy interchange of membranes so that membranes with a range of thickness and flexibility may be experimented with. Membranes are tightly sealed with o-rings in the pressure chambers, so no fluid would be expected to leak from the device. This would prevent excess use of fluid and as a result decrease the cost of experimentation. The membrane holder is also a reliable mechanism for securing membranes. In addition, design one requires a relatively simple fabrication that would decrease the production time.

Disadvantages

Although the device would be easy to fabricate, it may be difficult to assemble in a sterile environment, such as in the limited space of a cell culture hood. The pressure chambers may also be unstable in the wire stands, and loss of fluid or cell culture will not be tolerated. Despite the advantages this device offers, the disadvantages show it would not fully meet the design specifications.

Design Two

Design two features a membrane holder in which the membrane is situated between two plastic o-rings of different diameters. Specifically, the outer o-ring fits tightly around the inner o-ring with the membrane secured in between. Then, metal clips are fastened around the two o-rings to ensure that the seal holds. In this manner, the specific membrane utilized by the researcher can be removed from the holder to facilitate microscopy following experimentation as well as interchanged to allow for investigation of other options of membrane material (Figure 5).

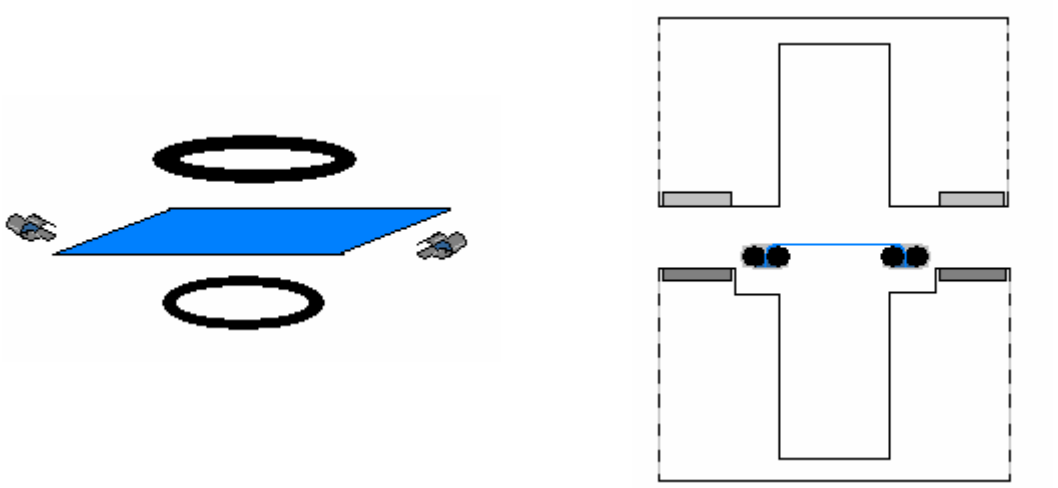


Figure 5. a. Design two membrane holder composed of two o-rings fastened around the membrane with metal clips. b. Cross section view of the upper and lower pressure chambers secured around the membrane holder by a metal-to-magnet connection.

During testing, the membrane holder is placed into the lower pressure chamber, modeled after a Nunc™ 4-well cell culture plate. Specifically, the membrane holder is positioned on a ledge within the wells that matches the total width of the o-rings. In this manner, as little of the o-rings as possible is exposed to the fluid pressure and the path through the membrane is undeterred. Then, the upper pressure chamber, which mirrors the bottom chamber with the exception of the recessed ledge, is situated to complete the system. The connection between the upper and lower chambers is made with a magnet-to-metal attraction in which metal plates are inlaid into the upper chamber and the corresponding magnets of similar size are inlaid into the lower chamber. Because of the square orientation of the four wells within the pressure plates, the fluid input and output pathways are positioned at a 90 degree angle, perpendicular to the outer walls of the plates (Figure 6).

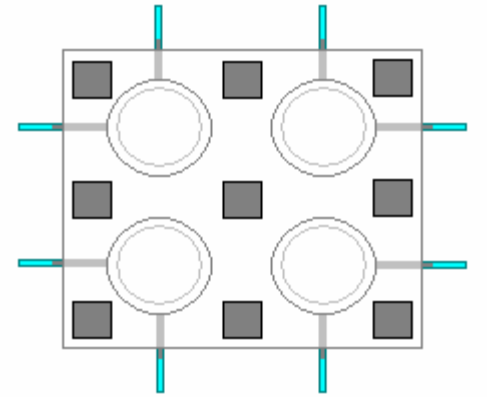


Figure 6. Plan view of the pressure chamber arrangement. Syringe input is depicted as the blue tubing on the left side of each chamber, while transducer output is on the right side of each chamber.

Advantages

Strong characteristics of design two include the fact that the experimental wells are oriented in a system of plates and are consequently much more stable on any working surface. Thus, the system is much more likely to withstand any accident contact, potentially avoiding the wasted time and revenue experienced with ruined experiments. In addition, due to the magnet-to-metal connection between plates as well as the design of the membrane holder, the system as a whole is very simple for the user to assemble. Specifically, instead of attempting to fit and rotate small parts to clamp the membrane into place as with design one, the membrane can be sealed within the gasket by fitting one o-ring around the other with one simple motion. In addition, assembly of the system can be completed

merely by lining up the metal plates and inlaid magnets, and no additional twisting of parts is required to fit the upper and lower pressure chambers together. This fact is especially important if experimental set-up must take place in a confined space as in a sterile fume hood.

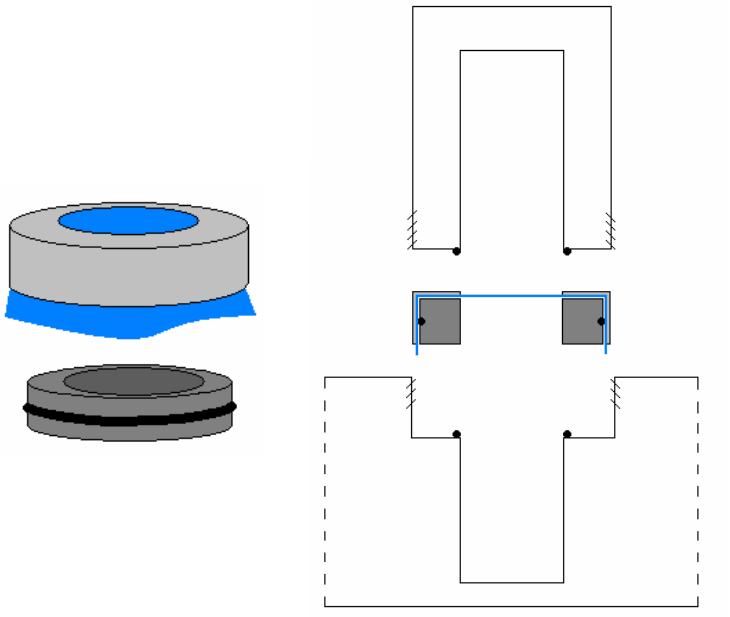
Disadvantages

Negative aspects of the design two include the fact that the magnet-to-metal connection, if not strong enough in comparison to the fluid pressure, may allow for leakage between wells. Also, because all of the wells are positioned within the same plate, if an error was to occur or adjustments were required in an individual well, the entire experiment would have to be interrupted to address the problem. In addition, because of the square orientation of the wells within pressure chambers, the distances between the wells and the fluid inflow from the syringe pump are different. Consequently, unless additional lengths of tubing are employed, the pressure would be administered unequally among wells. However, this additional tubing would serve only to complicate the system and make the design unnecessarily cumbersome.

Final Design

Our final design combines aspects of designs one and two with revisions to better meet the specifications of our client. First, the cell culture membrane will be held between a small ring-shaped magnet and an outer metal shell. We plan to use nickel-coated, axially magnetized neodymium ring magnets with a one inch outer and one-half inch inner diameter. Each ring has a magnetic field of 3665 Gauss and a pull force of 38.7 pounds (Amazing Magnets, 2007). The outer metal shell will be approximately one millimeter in thickness and will be constructed from nickel due to the metal's ferromagnetic properties and clean finish. The strong attraction of the membrane holder will be sealed with an o-ring surrounding the magnet in order to prevent fluid leakage (Figure 7).

The membrane holder will be placed in the lower pressure chamber atop an o-ring seal. The lower pressure chambers will likely be machined from cast acrylic designed for wash-down applications and high chemical resistance (McMaster-Carr, 2007). However, alternative materials are being investigated that could be sterilized with heat rather than with chemicals or antibiotics. The lower pressure chambers will be linearly arranged to allow consistent fluid flow and pressure transduction without the use of excess tubing (Figure 8).



a. b.

Figure 7. a. Neodymium 1" diameter ring magnet and nickel shell of the membrane holder. b. Cross-section of the upper and lower pressure chambers housing the membrane holder. O-rings are used in conjunction with threading of the chambers to seal fluid flow and prevent leakage.

Each lower chamber will be fitted with a cylindrical upper chamber by a threaded connection. Tightening of the thread will provide force to seal the membrane holder with a third o-ring. The individual upper pressure chambers will provide independence of each replicate and greater control over the sealing of each pressure chamber. The syringe input and transducer output ports will be located opposite one another in each upper and lower pressure chamber.

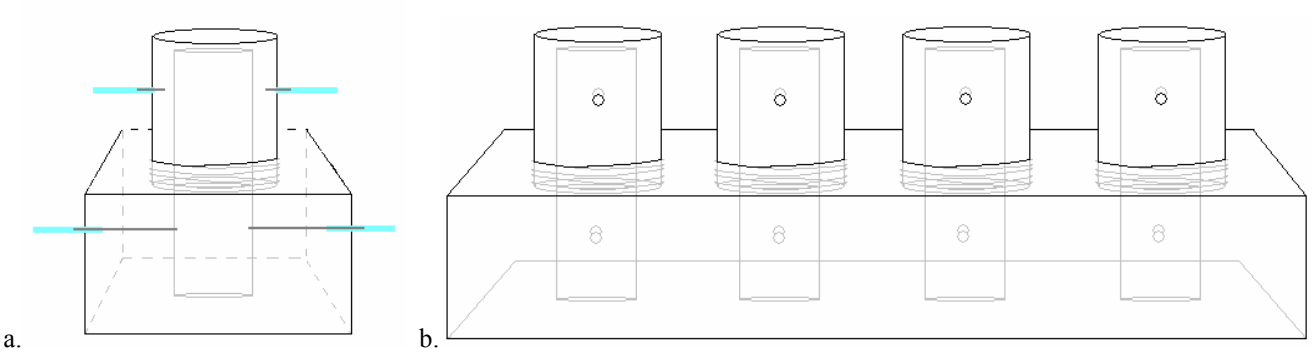


Figure 8. a. Side view of the device, including the syringe input and transducer output in each upper and lower chamber. b. Illustration of the linear arrangement of the pressure chambers that will facilitate a consistent flow of fluid into each chamber.

Advantages

Advantages of the final design include a strong sealing force that prevents fluid leakage across the membrane. Incorporation of multiple o-rings in each chamber also serves to prevent fluid leakage. Due to the individual upper pressure chambers and the linear arrangement of the assembled device, experimental replicates will be independent and performed with equal fluid pressure. The single bottom chamber will facilitate easy assemblage of the device and will therefore minimize user interaction. The final design will incorporate these components to fulfill the design specifications.

Disadvantages

At this time, the final design does not incorporate a mechanism for upper chamber pressure regulation. It has not been determined whether the device is required to maintain an upper pressure of precisely 18 mmHg. Therefore, this component will be further investigated in the future if necessary. Due to incorporation of magnets and nickel, a coating technique must also be determined that will prevent corrosion of these components in the fluid or in the chemicals used for sterilization. Epoxy was suggested by a representative of Amazing Magnets, and the chemical and heat properties of epoxy are currently being researched.

Design Matrix

To select a final design from the detailed alternatives, the effectiveness of each design was quantified based upon criteria that modeled the design specifications (Appendix A). The criteria included reliability, or the dependability of the design in terms of successful completion of experiments; user interaction, for which the less there is the better to avoid potential contamination; ease of use, so that whatever interaction there is for the user, it is simple for them to perform; replicate independence, in that if error is experienced or adjustments must be made to one well, they can take place without compromising the others; and finally, ease of manufacture because, as the device will be used in a laboratory setting, it must be precisely made. The criteria were ranked, with reliability and user interaction each receiving 25%, ease of use and replicate independence receiving 20%, and ease of manufacture representing 10% of the total rank. Then, each design was rated on a scale of 1 to 5, with a score of 5 indicating that the design best met the criteria.

The third design alternative obtained the highest overall score (3.7) due to characteristics that contribute well to reliability, user interaction, replicate independence, and ease of manufacture. Consequently, we believe this design will most effectively meet the needs of our client, and it is this design that we will construct as a final prototype.

Estimated Budget

The estimated budget for construction of the final design is 100 dollars. Materials included in this estimation are two 0.5 x 12 x 12 inch Plexiglas sheets for 50 dollars (Professional Plastics, 2007), four neodymium ring magnets at a cost of 20 dollars for 25 magnets (Amazing Magnets, 2007), and small hardware accessories for 30 dollars. Epoxy will be purchased and the magnets coated; however, Amazing Magnets also offers this service for approximately 50 dollars per magnet.

The final design depends heavily on construction rather than materials. Therefore, the estimated budget is well below the allocated funds of 2000 to 3000 dollars. This allocation was determined by Dr. Peters based on the 500 dollar cost of the current device constructed by the University of Wisconsin-Madison Department of Physics.

Future Work

Research is being conducted to determine whether back pressure regulators are necessary to maintain the upper chamber pressure of 18 mmHg (Figure 9). A back pressure regulator may be incorporated into each upper chamber and would contribute an additional 80 to 600 dollars to the expected budget, depending on the regulator model.

Figure 9. Miniature back pressure regulators (Omega, .2007)

Following this determination, materials will be ordered and construction begun. Experimentation will be especially important to optimize membrane sealing and fluid flow through the device. A completed device will be delivered at the end of the fall 2007 semester.

If the budget is within the allocated funds, additional pressure transducers may be purchased so that a greater number of experimental replicates could be performed. Additional funds may also be used to experiment with various cell culture membranes that could be incorporated into the device.

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Appendix A: Design Matrix

Criteria	Rank	Design One	Design Two	Design Three
Reliability	0.25	2	3	4
User Interaction	0.25	1	3	4
Ease of Use	0.20	3	1	2
Replicate Independence	0.20	4	1	4
Ease of Manufacture	0.10	3	2	5
Total	1.00	2.50	1.74	3.7

Appendix B: Product Design Specifications

Function

The perfusion chamber will allow for control of the movement of fluid across human eye cells that are adhered to an elastic membrane. Variable pressure is to be applied from both the top and bottom of the cells. The device must allow for adherence of the elastic membrane to the culture plates, easy replacement of cell culture plates, and measurement of fluid pressure with computer-controlled transducers. In addition, a porous elastic membrane that permits fluid flow will replace the silicon membrane of the current system. A successful design will be used to screen for potential treatments of glaucoma.

Client Requirements

The prototype must be modified or redesigned to:

- Allow for simultaneous experimentation on three samples of human eye cells.
- Apply pressure to both sides of the permeable membrane supporting the cells.
- Incorporate a more appropriate permeable elastic membrane.
- Reduce the amount of serum required for experimentation.
- Increase contact of cells with oxygen.
- Prevent fluid leakage.
- Simplify the process of sterilization or incorporate inexpensive disposable materials.
- Allow for easy exchange of cell culture plates.

Design Requirements

Physical and Operational Characteristics

a. *Performance requirements:* The perfusion chamber prototype will be used to investigate the effect of varied fluid pressure on the extracellular matrix proteins of ocular cells to further glaucoma

research. Consequently, the device must be designed with enough compartments to conduct triplicate experiments simultaneously. These wells must withstand pressures of forty millimeters of mercury without causing leakage or structural failure and should hold around five hundred microliters of cell culture media and drug treatment. Additionally, the system will be enclosed to deter contamination of the exposed cells when the device is in use. To provide a manner of measuring pressure and facilitating use, the developed system will be fully integrated with pre-existing technology, including an automated syringe pump with which to generate pressure, a pressure transducer to provide the output readings, and the corresponding software for data collection. Finally, the system must be easily disassembled to allow for sterilization and then easily reassembled for overall ease of use.

b. *Safety:* Safety necessities to keep in mind are those preserving the health of the cell cultures. To prevent bacterial contamination of the experimental and laboratory environment, maintenance of the seal between the porous membrane and the individual wells is imperative.

c. *Accuracy and Reliability:* To ensure reliability of the research conducted with the perfusion chamber device, the designed prototype must be correctly integrated into the current system through connection with the pressure transducer, accurate to 0.5 mmHg, syringe pump, and corresponding software. Error in this integration could result in unreliable data collection and misinformed interpretations of results. In addition, the design of the treatment wells must maintain the separation and independence of individual conditions to ensure reliability of potential findings.

d. *Life in Service:* The perfusion chamber design must endure weekly experimentation of continuous twenty-four hour periods for at least five years. Consequently, the device must be constructed of material that can withstand frequent gas sterilizations and treatment with antibiotics. Also, the structural integrity of the design must endure weekly resistance to fluid pressure.

e. *Shelf Life:* The product will be stored on the laboratory bench in a controlled environment when not in use. The pressure source and transducers may be disconnected when not in use.

f. *Operating Environment*: The chamber must withstand up to 40 mmHg fluid pressure and 2.5 μL per minute rate of fluid flow. It must be resistant to the drugs perfused into the cell culture and to the antibiotics, UV light, and gas used for sterilization.

g. *Ergonomics*: Operator-controlled components must be easily reached on a typical laboratory bench. The system assembly and exchange of cell culture plates must be simplified for the operator. Improved ergonomics may contribute to greater ease and speed of experimentation.

h. *Size:* Product size should be minimized to facilitate use with other equipment in the laboratory, including the pressure source, pressure transducers, and a computer.

i. *Weight:* Product weight should be minimized to allow easy transport within the laboratory and possibly transport by vehicle to the UW School of Medicine.

j. *Materials*: Materials utilized must be sterilized using UV light according to the client's current sterilization procedure. Plexiglas was suggested as the primary material for prototype construction.

k. *Aesthetics, Appearance, and Finish*: A transparent prototype will maximize visibility of the experiment.

Production Characteristics

a. *Quantity*: One prototype is needed that allows for triplicates of sample experimentation.

b. *Target Product Cost*: The client will determine the final budget at a later stage of the design. The preliminary budget allowance is $2000, estimated from the cost of manufacturing the Plexiglas container that is currently in use.

Miscellaneous

a. *Standards and Specifications:* The product is not required to meet any national or international standards. All specifications are determined by researchers in Dr. Peters's laboratory.

b. *Customer:* Proper function and ease of use are the customers' primary concerns.

c. *Patient-related concerns:* The device is not for use with human or animal subjects.

d. *Competition:* Perfusion chambers with similar capabilities exist but require whole eyes rather than eye cell cultures. To our and Dr. Peters' knowledge, the product will be a novel device.