Final 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. Testing in Dr. Peters's laboratory showed this design to meet the criteria of decreasing fluid leakage, being compatible with the existing technologies, allowing for at least three experimental replicates, and supporting a 4 μL per minute fluid inflow rate. Future work will focus on rigorous testing of the device during experimentation as well as identification of an ideal cell culture membrane. Following this work, it is hoped that this device may be useful to a variety of cellular-level research applications.

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Problem Statement

The goal of this project is to design and construct a device that measures fluid flow across a monolayer of trabecular meshwork cells adhered to a membrane. The membrane must be sealed within the device, with a fluid pressure of 18 mmHg maintained above and 30 mmHg maintained below the membrane.

Introduction

Overview of Glaucoma

Glaucoma is the 2nd leading cause of blindness in the world and affects approximately three million Americans, half of whom are unaware of their condition (Peters, 2007). These statistics highlight the importance of detection and treatment of glaucoma. The disease causes 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 normal vision (Figure 1).

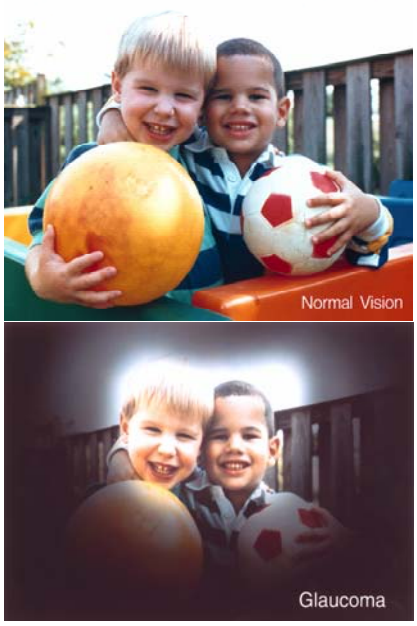


Figure 1. Comparison of normal vision and the vision of persons with glaucoma (National Eye Institute).

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). However, these ducts are partially blocked in an eye affected by glaucoma (Figure 2). The result is a buildup of ocular fluid in the anterior cavity of the eye and an increase in intraocular pressure (Hill *et al.* 2004).

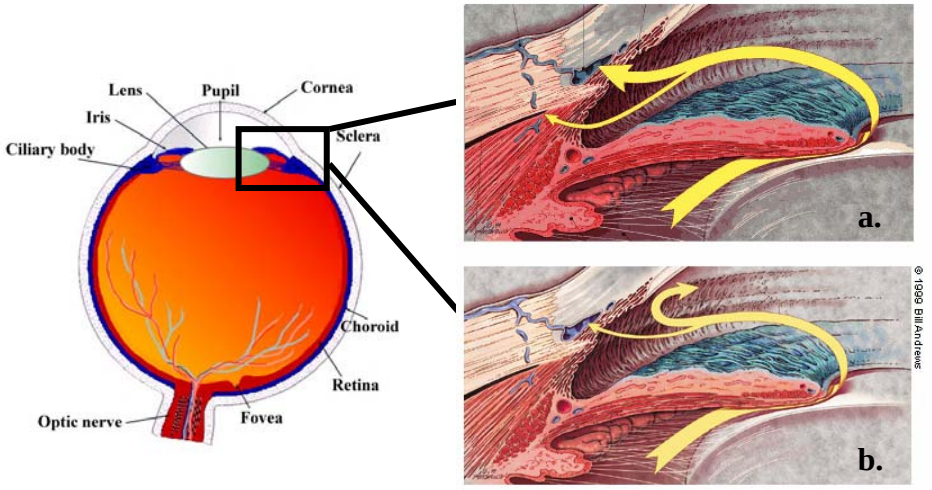


Figure 2. a. Fluid flow in a normal eye through the trabecular meshwork and uveoscleral ducts. b. Blockage of the ducts caused by glaucoma (Modified from Lewis *et al.*, 1999).

Current Treatment

The main goal of glaucoma treatment is to lower the intraocular pressure of the eye (Figure 3). This is accomplished by either decreasing the amount of fluid produced by the eye or by increasing the amount of fluid exiting the eye through the trabecular and uveoscleral ducts (Eye Digest, 2007). Eye drops are 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).

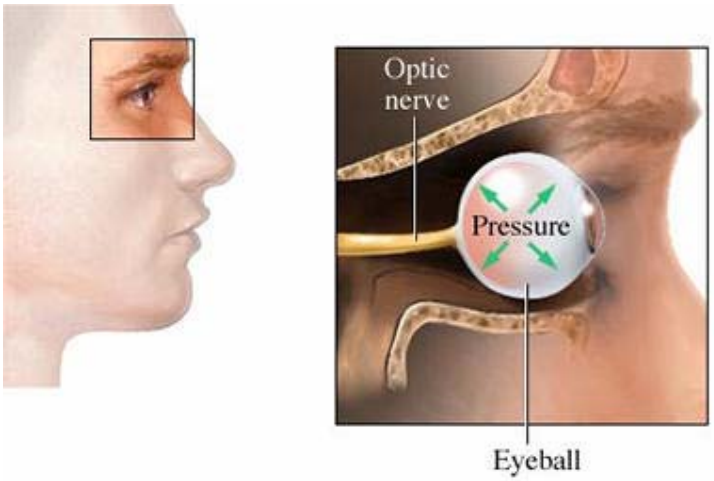


Figure 3. Images of the eye demonstrating the direction of the intraocular pressure (ehealth MD, 2007).

With this in mind our client, Dr. Peters, is working to develop a treatment to be injected directly into the eye that will increase the amount of fluid exiting the anterior chamber 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 an extracellular matrix peptide, such as β-catenin or fibronectin, 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 treatment effectively signals breakdown of the cytoskeleton, the cells "ball up" and allow fluid to pass. Dr. Peters measures this fluid flow with ISOTEC® pressure transducers that relay information to a computer.

Dr. Peters's 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.

Dr. Peters currently uses a system that 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. The system has several flaws that Dr. Peters would like to improve upon with 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 hydrogels. The device must also have the ability to run triplicate experiments simultaneously, and it must be integrated with the existing technology that includes a Harvard Apparatus® PHD 22/2000 Syringe Pump, 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 greater than two years. It must be easily disassembled for UV sterilization and must be constructed of materials that will not corrode with exposure to the fetal bovine serum cell culture media or to the chemicals used for sterilization, including phosphate buffered saline and the antibiotic gentamicin. 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 by a strong compressive force on the membrane holder created by 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 so that compression of the o-rings seals the system against 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 (30 mmHg), while the upper pressure mimics atmospheric pressure normally experienced at the outer surface of the eye (18 mmHg). Pressure outflow from the upper and lower chambers are measured with two pressure transducers, expected to measure pressure within ± 1.5 perccent or 1 mmHg (Harvard Apparatus, 2004). Finally, the individual pressure chambers are supported with wire ring stands for stability during experimentation.

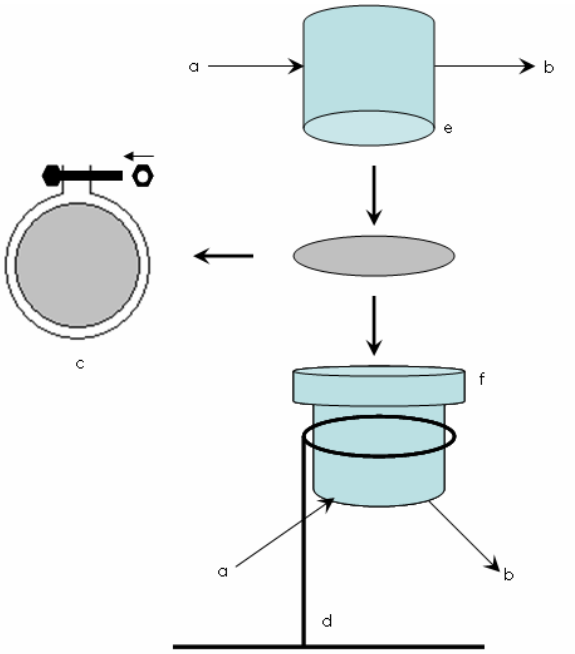


Figure 4. Design One. a. Fluid input from a syringe. b. Pressure measurement at a transducer. c. Membrane holder. d. Device stand. e. Upper pressure chamber. f. Lower pressure chamber.

Advantages

Design One allows for easy interchange of membranes to facilitate experimentation with a range of membrane thickness and flexibility. Fluid leakage is minimized by tightly sealing the membrane holder between the pressure chambers. This prevents excess use of fluid and a resulting

decrease in the cost of experimentation. In addition, each set of upper and lower chambers is independent, allowing for easy incorporation of additional replicates in the future.

Disadvantages

Although independent chambers are convenient, it may be difficult to assemble the experimental setup 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 must not occur. 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. Metal clips are then 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, and it can be interchanged to allow for investigation of other membrane material options (Figure 5).

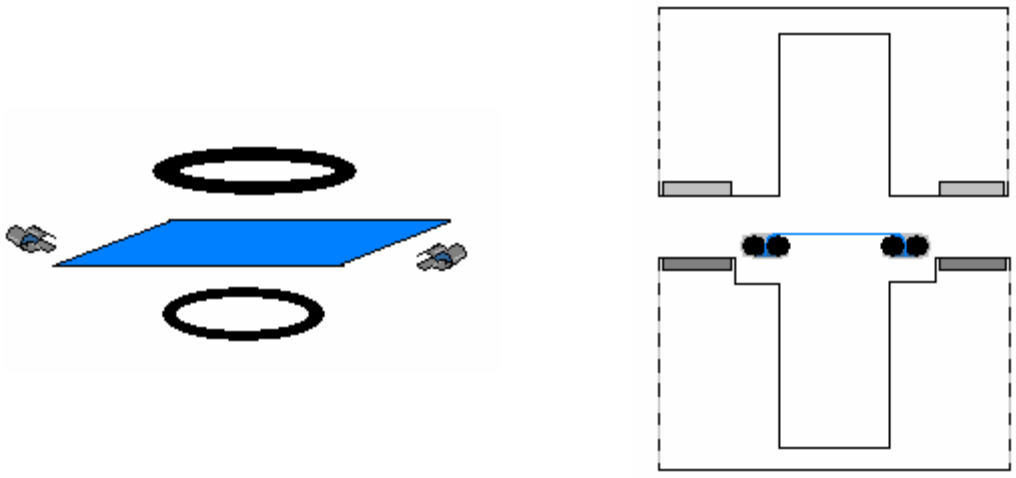


Figure 5. a. Design two membrane holder composed of two o-rings fastened around the membrane with metal clips. b. Cross section view of the upper and lower pressure chambers secured around the membrane holder by a metal-to-magnet connection.

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. The strong magnetic force of neodymium magnets compresses the upper and lower chambers around the membrane holder to seal against fluid leakage. 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 (Figures 6 and 7).

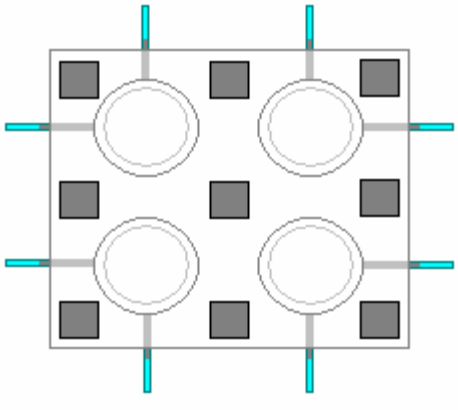


Figure 6. Plan view of Design Two. Syringe input is depicted as the blue tubing on the left side of each chamber, while transducer output is on the right side of each chamber.

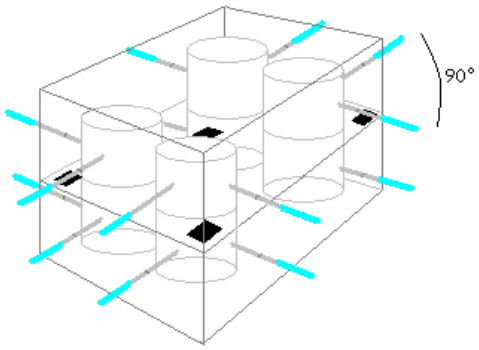


Figure 7. Side view of Design Two.

Advantages

Strong characteristics of Design Two include that the upper and lower chambers are attached in two plate systems and are consequently much more stable than individual chambers on a lab bench. This stability is intended to avoid the wasted time and revenue possible if experimental replicates are

overturned. 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 such as a sterile fume hood.

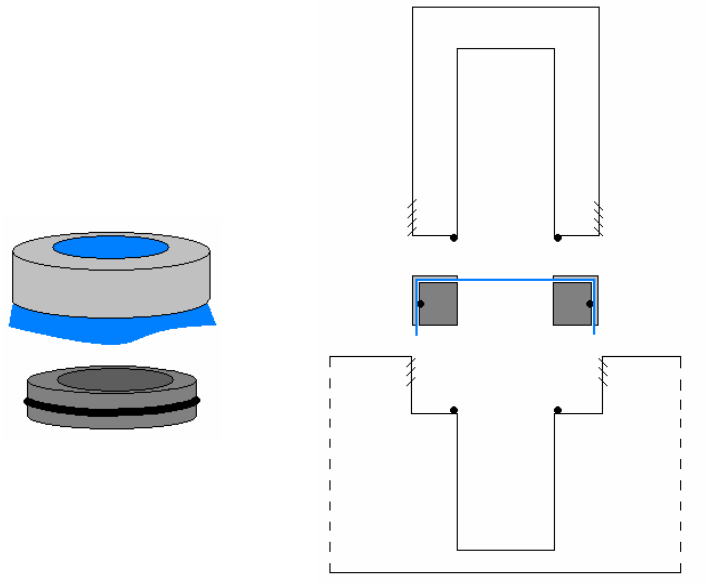
Disadvantages

Negative aspects of the design two include that the magnet-to-metal connection may allow for leakage between wells if the magnetic force is weaker than the force of the fluid pressure in the chambers. Also, because all of the chambers are positioned within the same plate, if an error was to occur or adjustments were required in an individual well, the entire experiment would need 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 not equal. Unless additional lengths of tubing are employed, the pressure would then be administered unequally among the chambers. However, any excess tubing would serve only to complicate the system and make the device unnecessarily cumbersome.

Design Three

Design Three combines aspects of Designs One and Two with revisions to better meet the specifications of our client. First, the cell culture membrane is held between a small ring-shaped magnet and an outer metal shell. The magnet is a nickel-coated, axially-magnetized neodymium

magnet. Each magnet has a magnetic field of 3665 Gauss and a pull force of 38.7 pounds (Amazing Magnets, 2007). The outer metal shell is approximately one millimeter in thickness and constructed from low carbon steel due to the metal's ferromagnetic properties and clean finish. The strong attraction of the membrane holder is sealed with an o-ring surrounding the magnet in order to prevent fluid leakage (Figure 8).



a. b.

Figure 8. a. Neodymium 0.5" 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.

The membrane holder is placed in the lower pressure chamber atop an o-ring seal. The lower pressure chambers are machined from cast acrylic designed for wash-down applications and high chemical resistance (McMaster-Carr, 2007). The lower pressure chambers are linearly arranged to allow consistent fluid flow and pressure transduction without the use of excess tubing (Figure 9).

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

a. b.

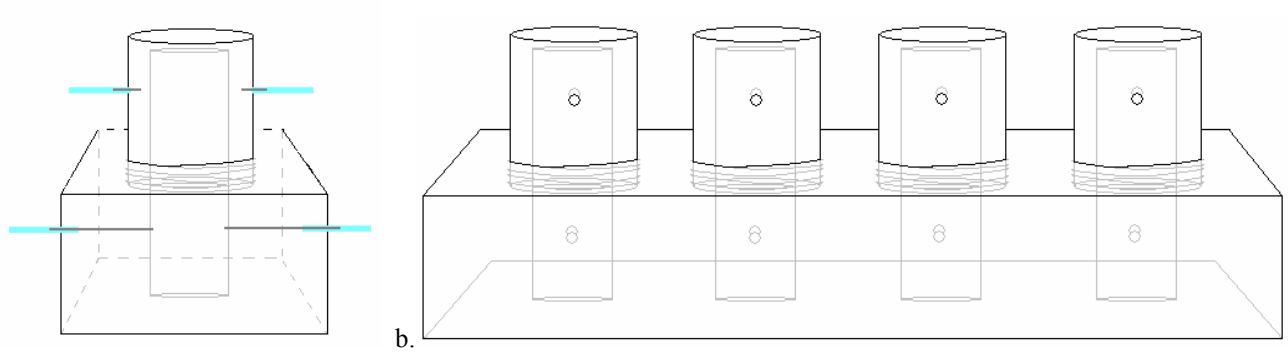


Figure 9. a. Side view of the device, including the syringe input and transducer output in each upper and lower chamber. b. Illustration of the linear arrangement of the pressure chambers that will facilitate a consistent flow of fluid into each chamber.

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 are independent and able to be performed with equal fluid pressure. The single bottom chamber facilitates assemblage of the device and therefore minimizes user interaction.

Disadvantages

Design Three 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 steel, a coating technique must also be determined that will prevent corrosion of these components in the cell culture media or in the chemicals used for sterilization. Epoxy was suggested by a representative of Amazing Magnets.

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, based on the ability of the device to support an appropriate fluid inflow rate and to apply pressures up to 30 mmHg; user interaction, for which the less there is the better to avoid potential contamination; ease of use by the device operator; 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. These 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 is expected to best met that criteria.

Design Three received the highest overall score (3.7) due to characteristics that contribute well to reliability, user interaction, replicate independence, and ease of manufacture. Specifically, Design Three has chambers that are tightly sealed for reliable fluid flow rate and pressure, is easy to assemble via a threaded connection of wells, incorporates four separate wells for replicate independence, and is expected to be relatively simple to manufacture. Consequently, we believe this design will most effectively meet the needs of our client, and it is this design that we constructed as a final prototype.

Final Design

The final design combines aspects of the proposed design alternatives in a manner that best satisfies the criteria outlined for the prototype, as determined by the rankings of the design matrix. Generally, the prototype is composed of four experimental wells oriented linearly with both upper and

lower pressure chambers. Between these chambers rests a membrane holder that secures a cell culture membrane between a metal encasing and a magnetic ring with o-rings employed to seal the assembly. Needle ports in each chamber serve as input connections to a syringe pump, allowing the infusion of fluid into the system and serve as outputs connections to pressure transducers for measuring the fluid released from the system.

Pressure Chambers

The four experimental wells of the final prototype are composed of both upper and lower pressure chambers, each with a 500 μL capacity as specified by our client. The small volume capacity will reduce the amount of media, and thus the expense, required for each trial. With upper and lower chambers, the device allows for subjecting of the cell layer to pressure from both above and below unlike Dr. Peters's current fluid column system that only allows for pressurization from above. As a result, experimental conditionss can more closely mimic the biology of the glaucomal eye, with the lower chamber set to elevated pressures around 30 mmHg and the upper chamber set to levels near air pressure, or 18 mmHg, normally experienced by the exterior of the eye. Additionally, by incorporating four wells, our client can not only conduct her experiments in triplicate, but she can also run a negative control of just a cell culture membrane with each trial. The information gained from the negative control could be especially useful in her research of an ideal porous and flexible membrane for her experimental protocol. The pressure chambers are machined from cast acrylic, a material chosen for its wash down applications and high chemical resistance. As cast acrylic is also the building material of the whole-eye chambers currently in use, this

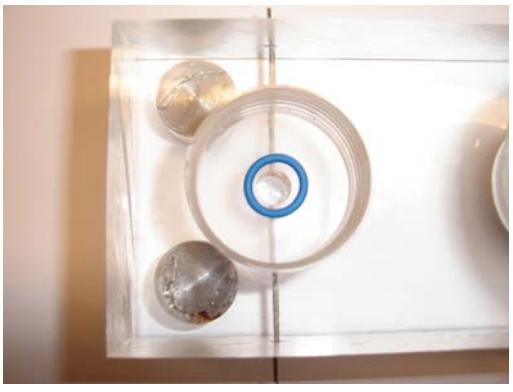


Figure 10. Top view of a lower pressure chamber.

material is known to be sterilizable with Dr. Peters's current methods of antibiotic treatment.

More specifically with regard to construction, the lower pressure chambers are oriented linearly within the cast acrylic base (9" x 2.25" x 1.25") (Figure 10). Similar to a well within commercial tissue culture plates, the chamber is cylindrical, with a diameter of 0.2814" and depth of 0.366". The chamber was milled into the acrylic at a total depth of 0.991", thereby allowing space for a 0.030" pocket to hold a plastic o-ring, and 0.620" for the membrane holder and the coarse threading that serves to seal the assembly.

An upper pressure chamber of the same dimensions was machined from a cast acrylic cylinder with a diameter of 1.75" and height of 1.5" (Figure 11). Similarly, a recess was created for an accompanying o-ring, again to a depth of 0.030" from the outer surface of the cylinder. For compatibility with the lower chamber, the bottom 0.5" of the cylinder was lathed to the diameter of the lower reservoir and coarsely threaded. The result is a compact set of wells that are easy to assemble and that ensure that each replicate remains independent of the others. Any event compromising an individual well will not ruin the entire experiment.

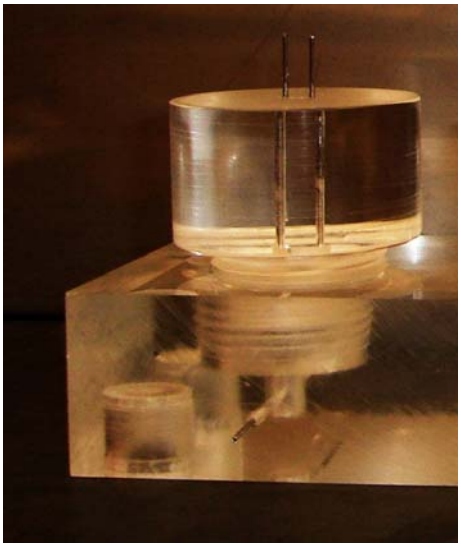


Figure 11: The upper pressure chamber, housed within an independent acrylic cylinder, is threaded into the lower chamber to complete assembly of the experimental well.

To allow for the infusion and outflow of fluid, 20-gauge surgical steel needles are drilled into each chamber. These needles, with a 0.0385" outer diameter and 1.5" length, are the same as those utilized in our client's whole-eye culture system and thus are compatible with the polyethylene tubing that connects the chambers to the existing technology in the

Peters lab. Specifically, the inflow ports connect to a Harvard Apparatus® syringe pump, and the outflow ports connect with ISOTEC® pressure transducers.

Finally, to ensure that the lightweight prototype is stable on a laboratory bench top, 0.625" diameter cylindrical steel weights were inlaid into the bottom 0.5" of the acrylic base, thus lowering the center of gravity of the system.

Membrane Holder

As our client has yet to identify the ideal porous and flexible cell culture membrane for her research, a membrane holder was constructed to be compatible with membranes of various materials and thicknesses up to 1.5 mm (Figure 12). Specifically, the membrane of choice is secured between a neodymium ring magnet (outer diameter: 0.5", inner diameter: 0.2814", thickness: 0.125") and a metal shell. Each magnet produces a field of 3665 Gauss and has a pull force of 38.7 pounds (Amazing Magnets, 2007). The metal encasing, with an outer diameter of 1.125", is composed of low-carbon steel, chosen for its ferromagnetic properties and easy machinability. At the center of the shell is a 0.2814" diameter opening, designed to provide a direct path between the cells seeded in this space and the pressure chambers of the same size. A 0.466" diameter recess was machined into the underside of the shell to hold an o-ring that seals the membrane between the magnet and metal, and a 0.625" diameter pocket was created into which the membrane and magnet fit. To prevent oxidation of the metal components of the membrane holder, these pieces were spray-coated with Performix® Brand Plasti-Dip®.

Figure 12: Components of the membrane holder, from left to right: the neodymium ring magnet, plastic o-ring, and the low-carbon steel encasing. The magnet and steel shell are coated with Plasti-Dip® rubber to prevent oxidation within the fluid system.

Implementation

Figure 13: Loading stand for easy assembly and disassembly for the membrane holder within a sterile hood.

To facilitate experimentation with the final prototype, a loading stand (2.75" x 2.25" x 1.25") was constructed with an magnet inlaid into a cast acrylic base (Figure 13). The membrane holder can thus be assembled on the base under a fume hood. The magnet is placed on the base, and the membrane of choice is laid over the magnet. Then, the steel shell, with an o-ring fitted into the recessed pocket, is positioned atop the membrane, with the attraction between the magnet and shell securing the membrane within the assembly. With a shearing motion, the membrane holder is removed from the base, followed by immersion in media within a petri-dish or cell culture well and seeding and growth of cells on the exposed membrane. Due to the compact size of the holder, it can be easily removed and placed under a microscope to determine when the cells have grown into a complete monolayer. At this point, an o-ring is fitted into the pocket created within the lower pressure chamber, the membrane holder is placed within the chamber, a second o-ring is positioned atop the steel shell, and the upper chamber is threaded into place to tightly seal the assembly (Figure 14). With the completed system, experimentation can take place, and following treatment, the membrane holder can be removed and disassembled again on the base or placed beneath a microscope for additional observation. Easy assembly

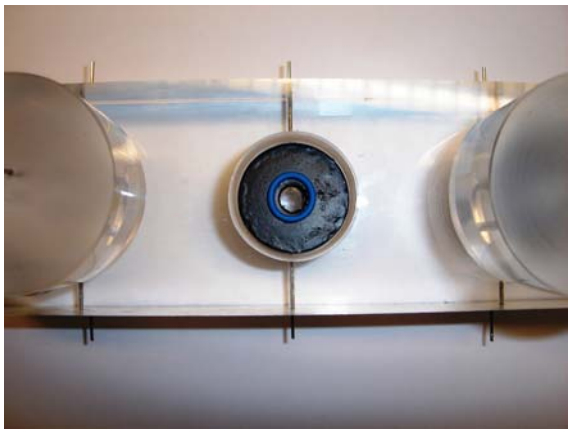


Figure 14: Assembled membrane holder positioned within lower pressure chamber. Atop the holder is an o-ring that serves to seal the membrane holder and the upper pressure chamber upon threading of the acrylic cylinder. Beneath the membrane holder is another o-ring that performs a similar function with the lower pressure chamber.

and disassembly are imperative, as all parts of the system are sterilized with antibiotic treatment 24 hours prior to testing.

Overall, our client's design specifications included criteria to allow for glaucoma research within a cellular-level perfusion chamber, as well as elements to facilitate this experimentation in Dr. Peters's laboratory. The final design incorporates components that address each of these established specifications with the goal of providing a well-functioning device that improves upon our client's current experimental set-up.

Prototype Testing

A series of tests was performed to determine whether the final design fulfills the established criteria (Appendix C). The membrane holders proved to be compatible with a variety of membranes ranging in thickness up to 1.5 mm. The device is also equipped to run up to four experimental replicates simultaneously. Testing in Dr. Peters's laboratory showed the prototype to be integrable with the existing technology, including a Harvard Apparatus® PHD 22/2000 Syringe Pump, ISOTEC® pressure transducers, and a computer. The device also requires minimal user interaction for setup and disassembly of the experiment. Additional quantitative tests investigated the sealing against fluid leakage and the ability to operate at an input rate of 4 uL/min.

Volume input and output of the upper chamber were compared to test the criteria of sealing against fluid leakage. The upper chamber was filled to capacity, and the syringe pump was set to an input rate ranging up to 40 μL per minute. Fluid was infused for 5 minutes, and the volume input was compared to the volume output from the chamber. Volume output was on average 92.39% of the volume input, corresponding to a maximum fluid leakage of 10 μL from a total volume of 700 uL. No fluid was observed to have leaked between the upper and lower chambers.

Linear correlation of the volumes of fluid entering and leaving the chamber was 0.9997, indicating consistent fluid flow at high and low input rates. In addition, 40 uL per minute is 10 times as great as the rate of fluid input expected during operation.

The results of testing support that the prototype significantly decreased the fluid leakage experienced with the previous device, is compatible with a variety of membranes, and is integrable with the existing technology. In addition, the device was shown to operate at and above an input rate of 4 uL/min. In conclusion, the series of tests determined that the prototype successfully achieved or exceeded each of these established criterion.

Future Work

Given these positive results, future work is planned to further investigate operation of the prototype in Dr. Peters's lab. The researchers in Dr. Peters's lab are looking to identify an ideal flexible and porous membrane for testing. After this membrane has been chosen, the device will be used to test the allowance of fluid flow across a monolayer of trabecular meshwork cells. The prototype will then be further investigated for performance during experimentation. A possible modification is incorporation of miniature backpressure regulators to more precisely control pressure in the upper chambers (Figure 15). Dr. Peters will determine whether this modification is necessary to achieve precise measurements of upper and lower fluid pressures. Finally, research is being conducted to identify a more appropriate coating for the metal and magnet of the membrane holder. The current Plastidip coating was not ideal for sterilization, and a professionally-applied polycarbonate or Teflon coating may improve both sterilization and operation of the device.

Figure 15. Miniature back pressure regulators (Omega, 2007)

Ethical Considerations

The success of our project required fabrication of a functioning prototype that fulfills all ethical considerations involved with the design. The primary ethical considerations included ensuring that the device would not interfere with the results of Dr. Peters's research and most importantly, ensuring the safety of the device operator. Specifically, the operator must be aware of the needles that protrude from each face of the prototype in order to avoid injury. In addition, the materials used to construct the device must be able to withstand sterilization and must not be affected by the cell culture media used in experimentation. This is important so that no parts of the prototype will not corrode or function less-optimally, especially in a manner that would negatively affect the outcome of Dr. Peters's research. Therefore, we considered the ethical consequences of all design decisions in order to engineer a prototype that is both safe and beneficial to Dr. Peters's research.

Conclusion

In summary, the prototype successfully meets the criteria of minimizing fluid leakage, being integrable with the existing technology in Dr. Peters's laboratory, allowing for up to four simultaneous experimental replicates, and operating at or above a fluid input rate of 4 μL per minute. The upper and lower chambers allow for application of varied pressure above and below the cells, which are set by the automated syringe pump to mimic the physiological environment of trabecular meshwork cells *in vivo*. Both the upper and lower chambers of the device have a maximum capacity of 500 μL, thus requiring significantly less fetal bovine serum than Dr. Peters's previous device. Additionally, space remains in the membrane holders to facilitate experimentation with membranes of variable thickness. Dr. Peters therefore has the option of interchanging membranes to identify the one most conducive to her research. Finally, the prototype is considered to be safe for the operator. All in all, the prototype

meets the design criteria discussed and provides an improved method for cellular-level glaucoma research. Following extensive laboratory testing, we hope this design may also be useful for a variety of cellular-level research applications.

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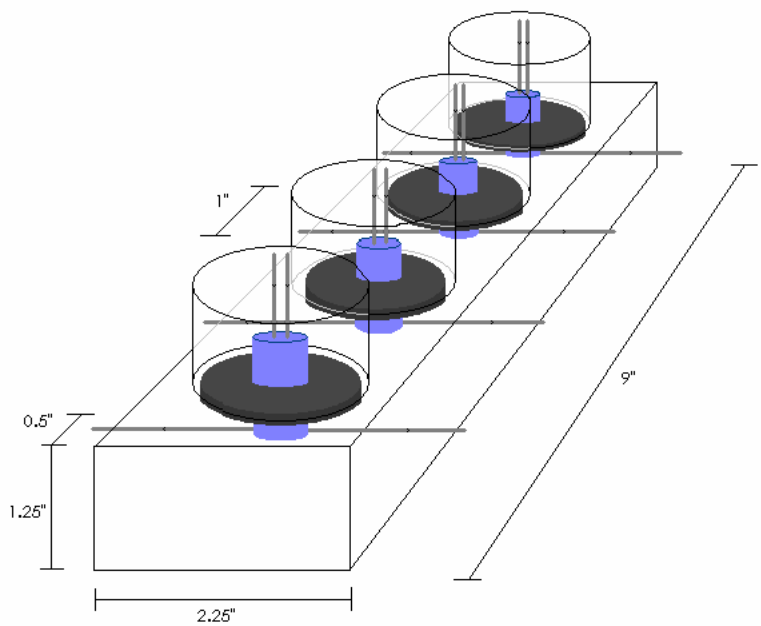
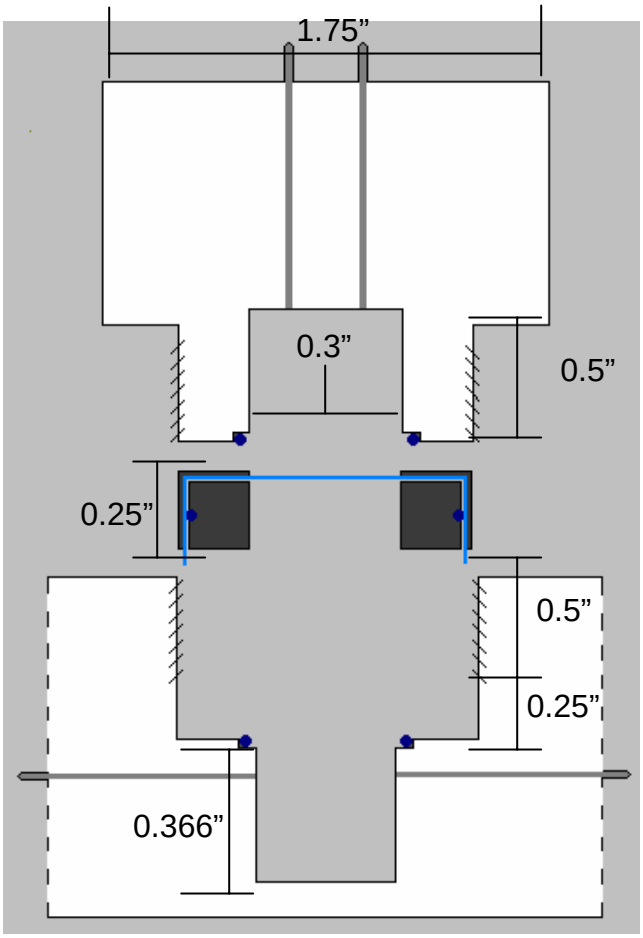
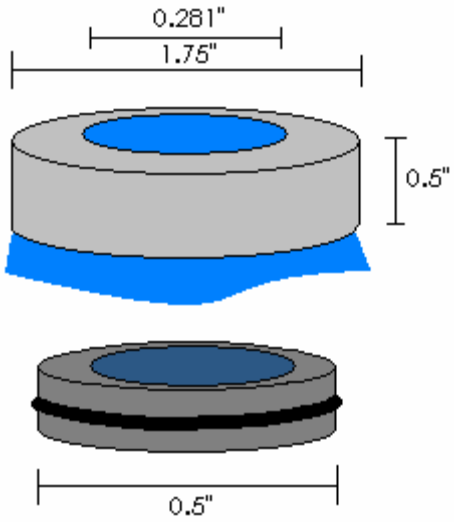
Appendix A: Design Matrix

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: Prototype Dimensions







Appendix C: Testing Results

Assessment of Fluid Leakage between Pressure Chambers:

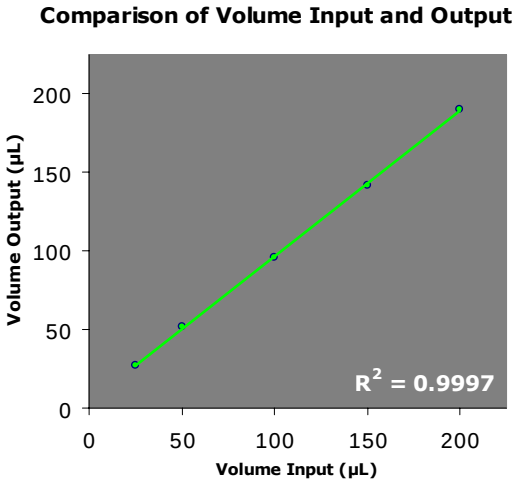


Figure 11. Comparison of volume input and output of the top chamber. Input for 5 minutes at a rate up to 40 L/min. Volume output determined from the mass of fluid collected.

Appendix D: Cost Analysis

The total project cost is well below Dr. Peters's expected budget of $2000 to $3000. The expected budget was based on the cost of a whole eye cell perfusion chamber designed and manufactured by the UW Physics Department. In addition, the total cost was only 20 percent the cost of Dr. Peters's current system.

Description	Vendor	Cost per Unit	Quantity	Cost
Plexiglas Rod (1.75"d x 1')	McMaster-Carr	$10.24	1	$10.24*
Plexiglas Sheet (12"x12"1.5")	McMaster-Carr	$39.03	1	$39.03*
Steel Rod (1.125"d x 1')	McMaster-Carr	$9.53	1	$9.53*
O-Rings (.5" ID x .28" OD)	McMaster-Carr	$2.56	4 packages of 5	$10.24*

NeFeB Magnets (.5" ID x .375" ID x .125")	Amazing Magnets	$11.00	1 package of 25	$11.00*
Needle Stock (20 gauge x 1')	Physics Shop	$0.00	2	$0.00
Plastidip	Home Depot	$10.00	1	$10.00
Sealing Glue	Dorn Hardware	$4.00	1	$4.00
Steel Rod (.625"d x 1')	Home Depot	$10.00	1	$10.00
			TOTAL	**$106.39**

*Cost does not include shipping and handling.

Appendix E: 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.