Perfusion Chamber for Cellular-Level Glaucoma Research

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Abstract

Glaucoma is a disease of the eye associated with increased pressure in the intraocular chamber resulting from a reduced fluid release through the ducts of the trabecular meshwork. The focus of current research is the identification of extracellular matrix peptides that may combat this accumulation, thereby reducing the pain and vision loss associated with glaucoma. The goal of this project is to construct a device with the capability to mimic the pressures experienced by trabecular meshwork cells in vivo so that peptides may be screened in cellular-level research. The device will control fluid pressures above and below the cell layer and integrate with pressure transducers that measure the release of fluid across a porous membrane. The final design incorporates a magnetic membrane holder for interchangeability and cast acrylic pressure chambers sealed to maintain pressures applied to the cell layer. Future development of the prototype will focus on precise regulation of pressure through additional regulators and identification of a flexible, yet porous membrane to allow for simultaneous utilization of both chambers.

Problem Statement

Goal: To design and construct a device to apply variable fluid pressures above and below trabecular meshwork cells seeded on a porous membrane

Background: Glaucoma is an eye disease characterized by elevated intraocular pressure, optic nerve damage, and progressive vision loss [3].

- Caused by blockage of normal drainage pathways through trabecular meshwork and uveolscleral ducts [1].
- Resulting buildup of ocular fluid in anterior cavity produces increased pressures and optic nerve constriction [1].

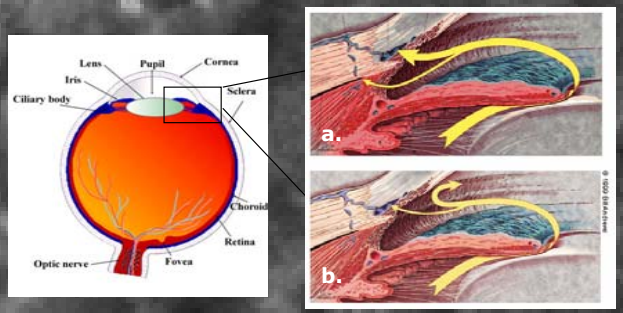

Figure 1: a. Fluid flow in a healthy eye; b. Blockage of flow pathways caused by glaucoma [2].

Problems with Past Systems:

- Whole eye culture system
  - Difficult to obtain whole eyes
  - Time-inefficient
- Fluid columns within tissue-culture plate
  - Frequent leakage
  - Expensive use of media

Client Motivation:

Goal 1: facilitate more efficient cellular-level research
- More replicated processed more quickly
- Immediate feedback on treatment effectiveness

Goal 2: identify peptides as potential glaucoma therapies
- More direct and effective treatment
- May lead to eventual cure for glaucoma

Design Criteria

The prototype must:

- apply fluid pressure up to 30 mmHg above and below the cell layer
- be compatible with various membranes
- run simultaneous experiments in triplicate
- integrate with existing equipment, including ISOTEC® pressure transducers
- be a sterilizable, closed system with minimal user interaction

Final Design

The final design is composed of four experimental wells with both an upper and lower pressure chamber. Between the chambers rests a holder that secures a membrane between a metal encasing and a magnetic ring with o-rings employed to seal the assembly. Syringe needles serve as input connections to a syringe pump, allowing the flow of fluid into the system, and outputs integrated with ISOTEC® pressure transducers for measuring the fluid released from the system.

Pictured at right, Cross-section of Pressure Chambers: The four assemblies of upper and lower pressure chambers are composed of cast acrylic. While the lower chambers lie within a continuous base to facilitate experimental set-up, separate upper chambers serve to individualize the replicates. Each well has a 0.5 mL volume and a threaded juncture forms the interface between the upper and lower chambers. Within each well is a recessed pocket that holds the o-rings which seal the entire system. Input and output of fluid for each well is conducted through 20-gauge, surgical steel syringe needles.

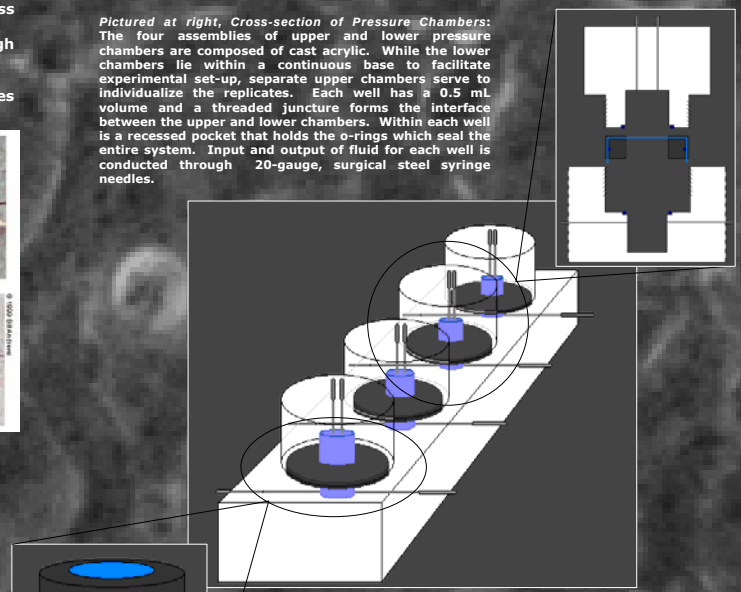

Figure 2: Diagram of Final Design

Pictured at left, Disassembled view of Membrane Holder: The membrane holder consists of an assembly of a low-carbon steel encasing and a neodymium ring magnet. It is the attraction between the axially-magnetized ring and ferromagnetic shell that holds the membrane tautly in place and allows for simple disassembly and interchange of various membranes. The pull force of this attraction is 38.7 lb. To prevent oxidation within the fluid system, these metal components are coated with a bio-inert liquid plastic. Plastic o-rings employed between the neodymium disk and steel encasing, as well as between the membrane holder and chambers, served to seal the system to prevent fluid leakage and contamination.

Prototype Testing

Assessment of Fluid Leakage between Pressure Chambers:

Volume input and output of the upper chamber were compared to assess fluid leakage within the system. The upper chamber was filled to capacity, and the syringe pump was set to inflow rates ranging up to 40 µL/min. This value is 10 times as great as the expected experimental rate. Fluid was input through one needle port for 5 minutes and simultaneously collected in a vial from the remaining port of the upper chamber. Volume output was determined from the mass of the filled vial and was on average 92.39% of the volume input, corresponding to a maximum fluid leakage of 10 µL and a maximum 7% error, both occurring at a rate of 40 uL/min. Linear correlation is 0.9997, indicating consistent fluid flow both at high and low inflow rates. No fluid was observed to have leaked into the lower chamber.

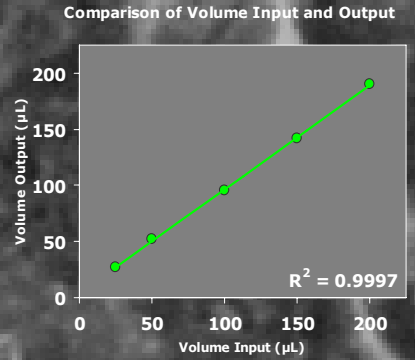

Figure 3: Comparison of volume input and output of the top chamber. Input for 5 minutes at a rate up to 40 µL/min. Volume output determined from the mass of fluid collected.

Future Work

- Incorporation of back pressure regulators to more precisely monitor chamber pressure
- Identification of ideal flexible and porous membrane

References

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[3] Peters, D.M. 2007. Use of cell-matrix interactions to treat glaucoma. Presentation. *University of Wisconsin-Madison, Department of Pathology and Laboratory Medicine.*

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Pictured in background: Cells of the human trabecular meshwork