THE HYDRODYNAMIC PERMEABILITY OF SCLERA

by

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Abstract

Previous studies have used several methods to determine the hydrodynamic permeability of sclera. In this study, the permeability of scleral tissue was measured via a direct perfusion system. A perfusion cell was designed, built and tested to measure the permeability of sclera samples. The experimental values were found to be in accord with the values determined by the other methods. The device was designed such that scleral samples would not be compressed more than 5 % of their undeformed thickness. To minimize confounding effects of blood vessels or aqueous veins in scleral samples, sclera from the posterior of the eye was used. Some tissue compression may be useful to collapse any vessels that might exist in the tissue. The experimental permeability measurements for bovine sclera were 3.6 x 10^{-14} cm^2.

Thesis Supervisor: Dr. Mark Johnson
Title: Principal Research Engineer

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Chapter 1

Permeability of Sclera

1.1 Introduction

Connective tissue is a classification for tissues in which a significant proportion of the tissue is composed of extracellular material. The composition of the extracellular material plays a large part in determining the special properties of the connective tissue. Providing both structural and defensive functions, connective tissues range from skin to cornea to cartilage. Connective tissue consists of two components, cells and extracellular matrix (ECM). The extracellular matrix is composed of fibers and an amorphous viscous ground substance (Gray 1970). The fibers include collagen and elastin. On the other hand, the ground substance is a non-fibrous element of the matrix, in which cells and other components are embedded. The highly viscous ground substance consists primarily of glycosaminoglycans and glycoproteins.

Since the characteristics of the connective tissue are dependent on the composition, changes in the composition with age is of special interest. Previous studies have individually examined the age-related changes of skin, lung, aorta, cartilage, cornea, and sclera tissues. The composition of the connective tissues does change with age. Some trends of the age-related changes include an increase in collagen as well as an increase in the cross-linking of the collagen (Kohn, 1978; Andreotti et al., 1983). Another trend is the decrease in glycosaminoglycans, most notably hyaluronic acid (Roberts and Roberts, 1973).

Although the general trends of age-related changes have been well documented, little effort has been devoted to determine the relationship between the age-related changes and the hydrodynamic permeability, or the specific hydraulic conductivity, of the connective tissues. The permeability of a tissue depends on its composition (Levick, 1987). Since tissue composition changes with age, one expects that tissue permeability might also change with age. Changes in the hydrodynamic permeability may have substantial consequences as the most tissues receive nutrients through the connective tissue space (the interstitium).

1.2 Purpose

The purpose of this work is to design and build an apparatus to be used to measure the permeability of small samples of connective tissue. After determining the permeability of a tissue, a biochemical analysis of the connective tissue can reveal the composition of the extracellular matrices. Changes in permeability can then be correlated to the changes in composition. By completing and comparing studies of several different connective tissues, the results can be used to predict the age-related changes of other tissues.

In particular, this study will examine sclera as a part of the investigation of the relationship between hydrodynamic permeability and age-related changes. This particular tissue has been selected for study for its ease of use and availability. Enucleated bovine eyes can be supplied from a local slaughter house while human eyes can be provided from the National Disease Research Interchange, along with the histories of the donors.

1.3 Calculation of Hydrodynamic Permeability

Hydrodynamic permeability (k) is an intrinsic measure of a porous material's flow conductivity. It is independent of the macroscopic length scales of the porous material and has units of cm^2. It is defined through Darcy's law:

$$Q = \frac{kA}{\mu h}\Delta P \tag{1.1}$$

where Q is the flow rate, μ is the viscosity of the fluid, A is the area perfused, h is the tissue thickness and ΔP is the pressure difference.

In what follows, the term permeability will to refer to the hydrodynamic permeability. Other terms have been used to characterize the flow conductivity of a porous material, including hydraulic conductivity and specific hydraulic conductivity. The hydraulic conductivity is the the flow rate per area over the pressure. Flow conductivity may also be written as L_p, where

$$L_p \equiv \frac{Q/A}{\Delta P} \tag{1.2}$$

The specific hydraulic conductivity($L_p\prime$) also takes the thickness (h) into account. The specific hydraulic conductivity can be calculated from:

$$L_p\prime \equiv L_p h. \tag{1.3}$$

However, the specific hydraulic conductivity can also be calculated from:

$$L_p\prime = \frac{k}{\mu}. \tag{1.4}$$

Thus, the permeability can be rewritten as:

$$k = \frac{\mu Q h}{\Delta P A} = \mu L_p\prime \tag{1.5}$$

1.4 Preliminary Studies

Several methods have been developed to measure the permeability of scleral and corneal tissue. (Corneal and scleral studies have often been conducted together as the availability, the concerns and the constraints are similar.) Hedbys and Mishima constructed a flow cell to measure the permeability of corneal tissue by direct perfusion

(1962). The flow cell consisted of two round filters made of porous stainless steel with solid steel rims. The filters were separated by a brass ring whose thickness could be adjusted for the tissue thickness. The entire assembly was clamped together, and the tissue was perfused with saline or water. From the experiments with bovine cornea, Hedbys and Mishima found the permeability of bovine cornea at 29°C to be approximately 1.9 x 10^{-14} cm^2.

Although the direct perfusion method works for the cornea, Fatt and Hedbys felt that this method would not work for sclera because the sclera is perforated (1970b): blood vessels in the sclera create holes in the tissue, and these holes are obviously more conductive to flow than the surrounding tissue. A hole present in the perfusion sample can significantly distort the permeability measurement. The method of measuring the scleral permeability must eliminate the effect of blood vessel holes to keep the measurements accurate.

In measuring scleral permeability, Fatt and Hedbys used the apparatus and procedure used by Fatt to measure the hydraulic conductivity of cornea (1968). With this method, the flow conductivity of water-swollen cornea tissue was calculated from the rate that the water left the tissue when compressed by a porous plate. Fatt and Hedbys measured the fluid conductivity of bovine and rabbit cornea and found the results to be in accord with Hedbys and Mishima's findings (1968,1970a).

Applying this procedure to sclera tissue, Fatt and Hedbys found the permeability for both human and rabbit tissues to be 1.9 x 10^{-14} cm^2, which corresponds to the permeability value found by Hedbys and Mishima (1962).

A third method to determine the permeability of sclera was used by Kleinstein and Fatt (1977). Kleinstein and Fatt measured the scleral permeability by applying a negative pressure through a special suction cup to the scleral surface. This method provided permeability measurements of 1.6 x 10^{-14} cm^2 for bovine sclera. These results concur with the results determined by Fatt and Hedbys' method. This method also provides evidence that capillaries have minimal effects on sclera permeability.

A study of scleral permeability using a direct perfusion method was conducted by Smith and Johnson (1984). Smith and Johnson measured the permeability of

anterior and posterior human sclera and found that the permeability values for the posterior samples were comparable to values from previous studies. As the tissue was under compressive load when the permeability was measured, it may be that tissue compression collapses any blood vessel that may be present.

However, Smith and Johnson also found anterior sclera to have a much higher permeability. They attribute the higher permeability to the aqueous veins. This suggests that either the posterior sclera is largely avascular, or more likely, any blood vessels present in the posterior sclera do not provide flow communication between the inner and outer scleral surfaces.

From a comparison of the methods and results of these studies, it was decided that the direct perfusion of posterior scleral tissue would be conducted in this study as the methodology is simple; an analysis of the potential effects of any blood vessel present will be presented in Chapter 4.

Chapter 2

Experimental Apparatus and Procedure

2.1 Apparatus

A direct perfusion flow cell was designed to measure the hydrodynamic permeability of sclera. In order to measure accurately the permeability of the tissue by direct perfusion, the apparatus must meet several design considerations. The perfusion apparatus must limit all flow to that through the tissue. Because the permeability for sclera is in the order of $10^{-14}cm^2$, the smallest leak will have an enormous affect on the permeability measurement. The apparatus must also slightly compress the tissue. By adding a compressive force to the tissue, any distortion to flow conductivity as a result of pores created by blood vessels can be eliminated. Compacting the tissue will cause the vessels to collapse and eliminate any flow channels across the tissue. Compaction is limited to five percent of the tissue thickness to minimize any effects on tissue permeability. Finally, the apparatus must be easy to assemble. With these considerations, the following design was developed. A comparison with existing designs will follow.

The perfusion system consists of the perfusion cell, a syringe pump, a pressure transducer and a data acquisition system. A schematic of the perfusion system is shown in Figure 2-1.

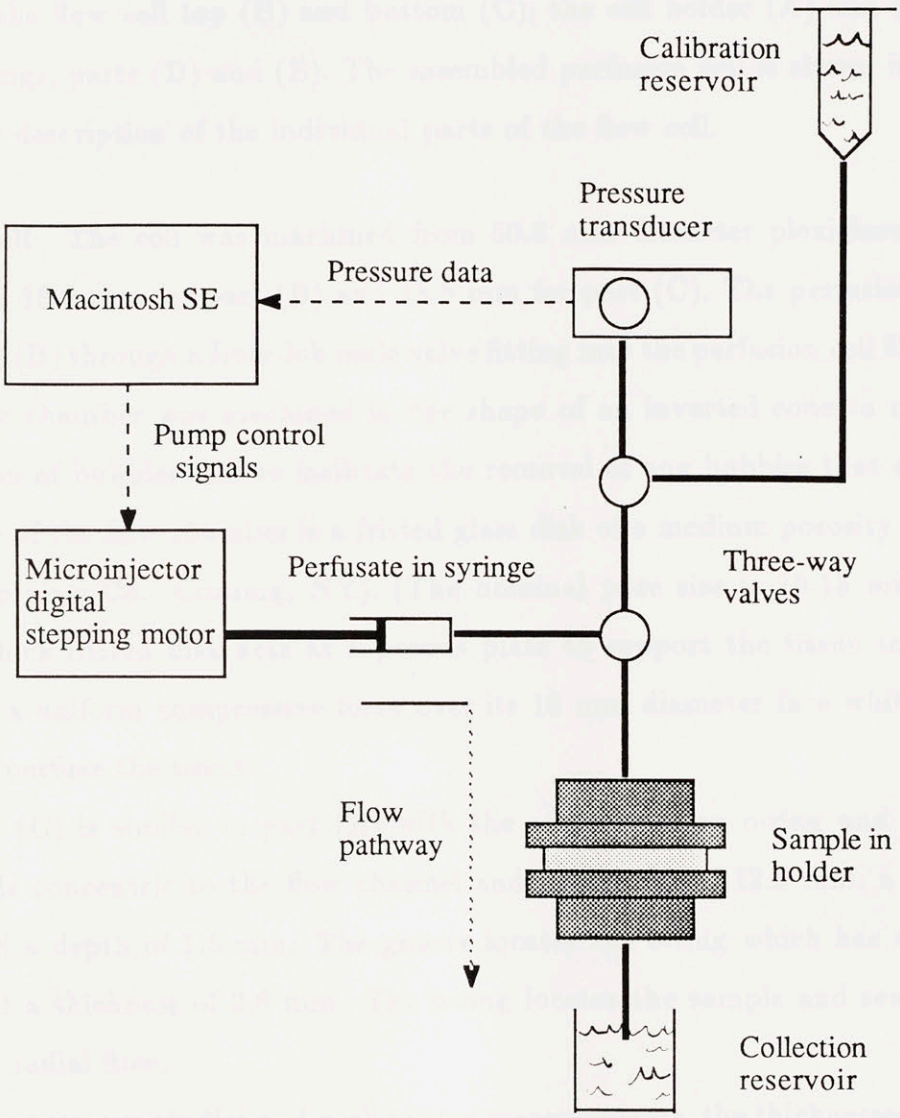


Figure 2-1: Schematic of perfusion system.

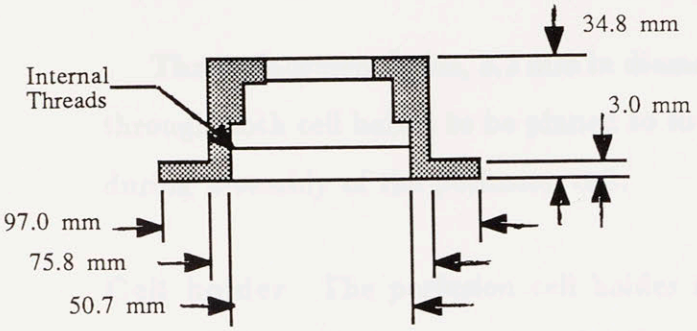
2.1.1 Perfusion cell

The perfusion cell is shown in Figure 2-2. The principal components of the perfusion cell are the flow cell top (B) and bottom (C); the cell holder (A) and (F); and the spacer rings, parts (D) and (E). The assembled perfusion cell is shown in Figure 2-3 after the description of the individual parts of the flow cell.

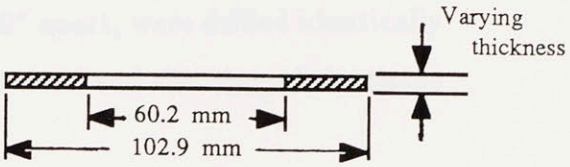
Flow cell The cell was machined from 50.8 mm diameter plexiglass, and has a height of 18.0 mm for part (B) and 18.5 mm for part (C). The perfusion fluid flows into part (B) through a Luer-lok male valve fitting into the perfusion cell flow chamber. The flow chamber was machined in the shape of an inverted cone to minimize the formation of bubbles and to facilitate the removal of any bubbles that did form. At the base of the flow chamber is a fritted glass disk of a medium porosity (part 31001-10M, Corning Co., Corning, NY). (The nominal pore size is 10-15 microns.) The 2 mm-thick fritted disk acts as a porous plate to support the tissue sample and to provide a uniform compressive force over its 10 mm diameter face while permitting fluid to perfuse the tissue.

Part (C) is similar to part (B) with the addition of an o-ring and groove. The groove is concentric to the flow channel and has an ID of 12.5 mm, a width of 3.5 mm and a depth of 1.5 mm. The groove locates the o-ring which has an ID of 12.7 mm and a thickness of 2.8 mm. The o-ring locates the sample and seals the cell to prevent radial flow.

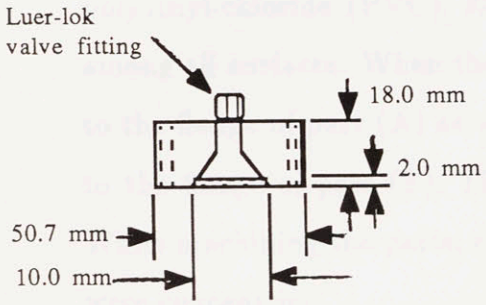
From previous studies and preliminary measurements, the thicknesses of the sclera tissue samples were found to range between 1.0 mm and 2.0 mm. To seal the flow cell, the o-ring selected must span the distance created by the tissue and compress against parts (B) and (C). From the dimensions of the o-ring and o-ring groove, the o-ring can seal for all tissues having a thickness of 1.25 mm or less. For tissue samples greater than 1.25 mm thick, a rubber gasket, with an ID of 13.1 mm, a width of 7.5 mm and a thickness of 0.8 mm, is placed under the o-ring. The gasket extends the sealing capabilities of the o-ring, allowing the o-ring to seal for the entire tissue range of 1.0 mm to 2.0 mm.



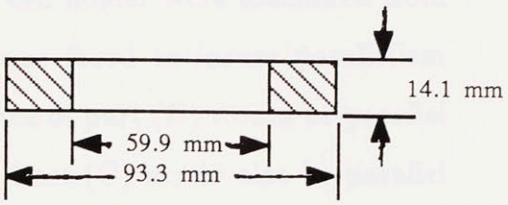
Part A. Cell holder top



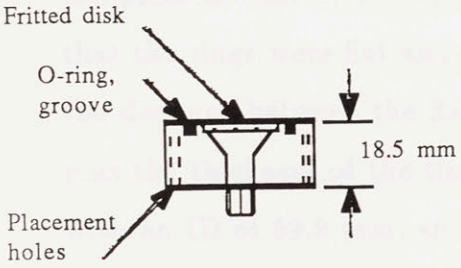
Part D. Variable thickness spacer ring



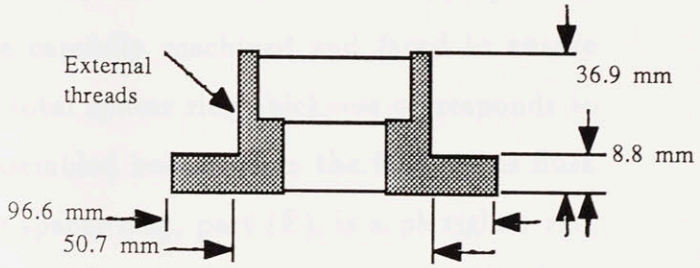
Part B. Perfusion cell top



Part E. Base Spacer Ring



Part C. Perfusion cell bottom



Part F. Cell holder bottom

Figure 2-2: The perfusion cell

Three placement holes, 3.2 mm in diameter and 120° apart, were drilled identically through both cell halves to be pinned to to prevent rotation and shearing of the tissue during assembly of the perfusion cell.

Cell holder The perfusion cell holder is shown as parts A and F. The flow cell halves fit into their appropriate holder. The top and bottom parts of the holder clamp and align the flow cell half. Both parts of the cell holder were machined from polyvinyl-chloride (PVC). Each half of the holder was faced to insure parallelism among all surfaces. When the cell is assembled, the face of part (B) would be parallel to the flange of part (A) as well as flush to part (C). Part (C) would also be parallel to the flange of part (F). Thus the flanges of parts (A) and (F) would be parallel. While machining the parts, care was taken to ensure that the center lines for all parts were concentric.

Spacer rings The spacer rings, Parts (D) and (E), are used to specify the distance between the two halves of the flow cell so that the tissue sample will be appropriately compressed. All of the spacer rings were carefully machined and faced to ensure that the rings were flat and parallel. The total spacer ring thickness corresponds to the distance between the flanges of the assembled holder when the flow cell is flush plus the thickness of the tissue. The base spacer ring, part (E), is a plexiglass ring with an ID of 59.9 mm, an OD of 93.3 mm and a thickness of 14.1 mm. The base spacer ring was implemented to decrease the amount of material needed to make part (D). Part (D) is an interchangeable PVC spacer ring of a ID of 60.2 mm and an OD of 102.9 mm. The part (D) spacer ring and its thickness varies with with each experiment according to the thickness of the tissue sample. The thickness of part (D) is comprised of the space allowed for the tissue plus the remaining space between the flanges. The range of the disks to be used as part (D) corresponds to the tissue thickness range of 1 mm to 2 mm. The thicknesses of the spacer rings are measured with an accuracy of 0.03 mm. Table 2-1. lists the spacer rings available and the appropriate tissue thicknesses for each ring. The difference between the tissue

Spacer ring thickness (mm)	Corresponding tissue thickness with out compression (mm)	Corresponding tissue thickness with 5% compression (mm)
3.07	0.82	0.86
3.43	1.18	1.24
3.47	1.22	1.28
3.58	1.33	1.40
3.89	1.64	1.73

Table 2.1: Spacer rings and tissue thicknesses

thicknesses with and without compression corresponds to the range of tissue thickness appropriate for each spacer ring.

Because the design of the holder uses screwing mechanism similar to a nut-and-bolt assembly, the clamping mechanism is separate from the spacer rings. The o-ring and tissue will be compressed as determined by the thickness of the spacer ring. The range of tissue compression is limited by the thicknesses of the spacer rings that are available. The estimate of the accuracy of the compression is +/− 0.03 mm. The accuracy takes into account the tolerance of the thickness of the variable spacer ring and variation of the flanges of the holder and base spacer ring, assuming all parts are flush when assembled. The assembled flow cell is shown in Figure 2–3.

2.1.2 Pressure Transducer

The perfusion cell is attached to a pressure transducer which monitors the pressure. The pressure transducer is a Microswitch Pressure Sensor (142PC05G, Brownell, Co., Woburn, MA) which can measure with an accuracy of +/− 0.5 % full scale output and can measure a maximum pressure of 259.3 mm Hg.

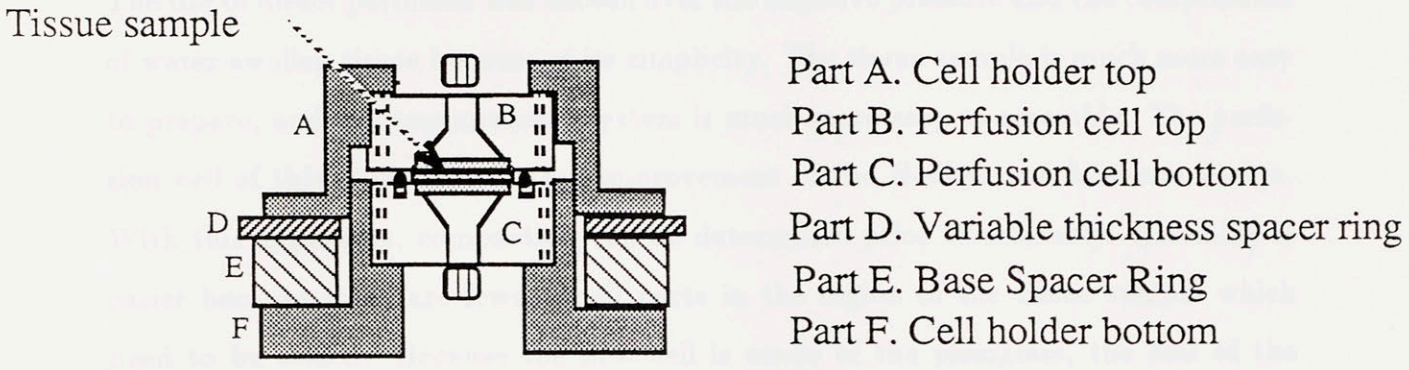


Figure 2-3: Assembled flow cell

2.1.3 Syringe Pump

The perfusion pump regulates the flow rate of the perfusion fluid. The pump, Syringe Infusion Pump 22, is manufactured by Harvard Apparatus (South Natick, MA). The pump may be run at a constant flow rate or at a constant pressure. Using a 250 μl syringe, the minimum measureable flow rate is 0.01 μl/minute. For the current experiment, the range of flow rates were from 0.01 μl/min to 0.20 μl/min after two hours of equilibration.

2.1.4 Computer

A Macintosh SE is the basis of the data acquisition system. The perfusion data is collected by and the flow rate is set by the computer. A perfusion program which uses a control loop, monitors and regulates the pressure and the flow, and then calculates the changes necessary to maintain the designated steady-state parameter.

2.2 Improvements of Previous Designs

The use of direct perfusion was chosen over the negative pressure and the compression of water-swollen tissue because of its simplicity. The tissue sample is much more easy to prepare, and the measurement system is much more easy to assemble. The perfusion cell of this study is a further improvement of the Hedbys and Mishima design. With this apparatus, compaction can be determined prior to assembly. Assembly is easier because there are fewer small parts in the region of the tissue sample which need to be sealed. Because the flow cell is made of the plexiglass, the seal of the o-ring against the flow cell is visible. Because of the threaded clamping mechanism, the holder can easily be connected and tightened.

2.3 Procedure

The sclera samples are taken from young bovine eyes, which were enucleated at a local slaughter house and stored in ice-saline. The sclera is excised after the extraneous muscle and conjunctiva are removed. The sclera is then separated from the cornea, lens, iris and retina tissues. The choroid and episclera are also carefully removed by scraping the tissue with a scalpel. Using a 12.0 mm trephine, a tissue sample is cut toward the posterior of the sclera. The sample was selected to have as few blood vessels and as uniform a thickness as possible.

The thickness of the sample is determined using a current-sensing micrometer, built following the design of Eisenberg and Grodzinsky (1985). The current-sensing micrometer was built from a voltmeter, a micrometer thimble, an aluminum plate and a plexiglass holder. The voltmeter's positive terminal is attached to the micrometer thimble, which is mounted on the plexiglass; and its negative terminal is attached to the aluminum plate, which rests inside the plexiglass. To measure the tissue thickness, the tissue is placed on the aluminum plate underneath the micrometer. The micrometer thimble is adjusted until the thimble reaches the sclera. Contact with the sclera is observed when the voltmeter registers a finite resistance. The

thickness of the tissue can then be read from the micrometer thimble. This method of measuring the sclera allows the thickness of the tissue to be determined without compression of the tissue.

To assemble the perfusion cell, the tissue sample is placed on the bottom half of the flow cell, part (C), which is resting in the holder bottom, part (F). The sample rests within the o-ring on the fritted disk of part (C). The fritted disks of both halves of the flow cell are wetted with perfusate (Dulbecco's phosphate buffered saline, Gibco, Grand Island, NY) to prevent the dehydration of the tissue, which will affect the permeability, and to reduce the possibility of bubbles in the system. The edges of the sample are peripherally sealed to the o-ring by a silicone rubber compound (Microfil, Canton Bio-Medical Products, Inc., Boulder, CO.). This lacquer seal should prevent fluid from flowing around and between the tissue and the o-ring. Part (B) is placed over the assembly and pinned in place through the placement holes.

To attach the holder top to the bottom with the desired compression on the tissue, the spacer rings must be placed between the two to act as an interference at at the specified height. Before attaching the holder top, the base spacer ring and the variable spacer ring are first placed on the bottom half of the holder. The variable spacer ring is selected, taking into account the tissue thickness and the desired compression, 1 – 5 % of the tissue thickness. With parts (B), (C), (D), and (E) in place, the cell holder top is screwed onto the bottom until the flanges of top and the bottom are flush to the spacer rings. Since the flow cell halves are pinned to each other, screwing the holder halves together only applies a compressive force on the tissue.

Using a needle-tipped syringe, the flow chamber of each half of the flow cell is filled with the perfusion fluid, taking care to avoid bubbles. The cell is then attached at the inflow to the perfusion pump and the transducer and at the outflow to the outflow reservoir, taking care to insure complete fluid connections. Once assembled, the perfusion cell is allowed to equilibrate for an hour. The sclera is perfused for five hours at a constant pressure of 18 mm Hg (intraocular pressure).

Chapter 3

Experimental Results

3.1 Control Experiments

Two control experiments were conducted to insure that the experimental flow cell fulfilled the design criteria. During the perfusion, the perfusate must flow through the flow chambers, the porous plates, and the tissue sample. The fluid may not leak between the two halves of the flow cell nor between the tissue and the o-ring.

For the first test, the system was assembled with a rubber disk as the tissue sample. The rubber disk, with a thickness of 1.35 mm, was cut by the trephine which was used to cut the tissue samples. Instead of using Microfil rubber silicone to seal the tissue to the o-ring, RTV 110 rubber silicone was used. The spacer ring was selected to compress the rubber 2.4%. Once assembled, the perfusion was run by constant pressure following the normal procedure. Upon reaching the designated perfusion pressure, the pressure then remained constantly high without flow (+/- 0.01 μl/min), as the rubber tissue sample prevented the flow from perfusing through the tissue. This experiment proves that the o-rings can effectively seal against the two flow cell halves. Thus the maximum flow resistance measurable by the current system is 18 mm Hg/(0.01 μl/min) which is equal to 1800 mm Hg$\cdot$ min/μl.

In a second preliminary test, a filter of a uniform 0.08 μm porosity was substituted for the tissue (Nuclepore PC MEMB 50MM, Pleasanton, CA). To seal the filter sample, the filter was placed between the o-ring and the gasket and sealed with RTV

110 silicone rubber. This procedure was selected because neither compressing the filter nor sealing the edges of the filter could adequately contain the flow. The cell holders were screwed together until the cell halves were flushed instead of using the spacer rings as none of the spacer rings were machined for a tissue thickness of 0 mm. In the perfusion apparatus, the filter was perfused with filtered, de-ionized water for one hour. With a constant pressure of 20 mm Hg, the flow rate was found to be about 71 μl/minute. From the definition of flow resistance, the resistance, R, can be found from:

$$R = \frac{\Delta P}{Q} \tag{3.1}$$

where ΔP is the pressure drop across the filter and Q is the flow rate. The resistance of the filter was determined to be approximately 0.28. This value corresponds to resistance value of a 0.08 mm filter as determined by Johnson in earlier studies of resistances of filters (unpublished).

3.2 Measurement of Sclera Permeability

A bovine scleral tissue sample was perfused using the experimental perfusion cell. From the current-sensing micrometer, the tissue thickness was measured to be 1.32 mm which prescribes a spacer ring thickness of 3.57 mm. From the available rings, the variable spacer ring thickness was chosen to be 3.58 mm. This spacer ring allowed the perfusion apparatus to perfuse the tissue uncompressed. Using a 20 mm Hg constant pressure perfusion, the resulting flow rate was found to be approximately 0.05 μl/min after approximately two hours of equilibration.

The plots of the pressure and flow rate as a function of time is shown in Figures 3-1 and 3-2. In the plot of the pressure against time, the variation of the pressure is caused by noise in the system as a result of the control loop of the perfusion data acquisition program. The noise of the pressure is also evident in the flow rate as the computer-controlled pump adjusted the flow to keep the pressure constant.

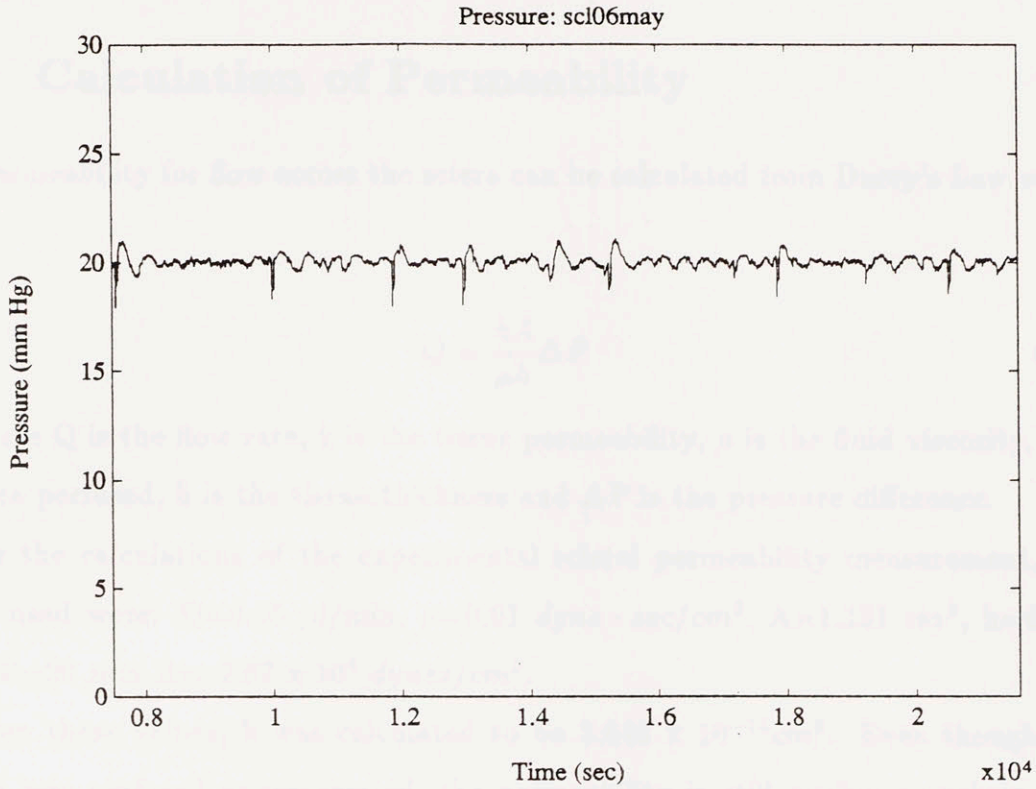


Figure 3-1: Pressure vs. Time plot of experimental permeability measurement.

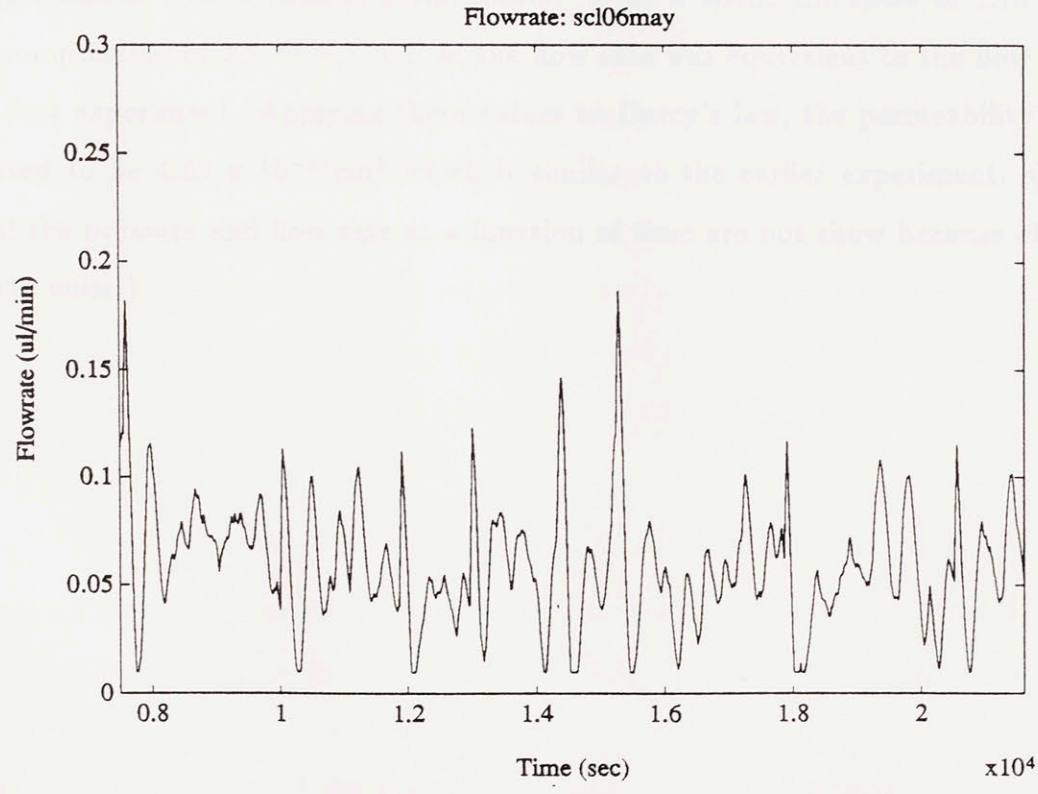


Figure 3-2: Flow rate vs. Time plot of experimental permeability measurement.

3.3 Calculation of Permeability

The permeability for flow across the sclera can be calculated from Darcy's Law which is:

$$Q = \frac{kA}{\mu h}\Delta P \tag{3.2}$$

where Q is the flow rate, k is the tissue permeability, μ is the fluid viscosity, A is the area perfused, h is the tissue thickness and ΔP is the pressure difference.

For the calculations of the experimental scleral permeability measurement, the values used were: Q=0.05 μl/min, μ=0.01 $dyne \cdot sec/cm^2$, A=1.131 cm^2, h=0.132 cm, ΔP=20 mm Hg=2.67 x 10^4 $dynes/cm^2$.

From these values, k was calculated to be 3.643 x $10^{-14} cm^2$. Even though the sample was perfused uncompressed, the permeability is still similar to values that previous researchers have found for scleral permeability.

A second experiment was conducted, and its permeability value concured with the experimental hydrodynamic permeability. With a tissue thickness of 1.70 mm and a compression of 3.5 % +/- 1.7 %, the flow rate was equivalent to the flow rate of the first experiment. Applying these values to Darcy's law, the permeability was calculated to be 4.69 x $10^{-14} cm^2$ which is similar to the earlier experiment. (The plots of the pressure and flow rate as a function of time are not show because of the excessive noise.)

Chapter 4

Discussion and Conclusions

Using the experimental apparatus to determine the permeability of sclera yielded a value that was similar to (although slightly different from) values from previous studies. Four mechanisms are discussed which may affect tissue permeability and may explain any discrepancy.

4.1 Flow Through Blood Vessels

A concern in measuring the permeability of sclera is the effect of blood vessels as an alternate flow path. If a blood vessel is present in the tissue sample, then the vessel may act as a pipe through the tissue. The effects of the blood vessel on the measurement of scleral permeability must be calculated to determine its significance.

The flow of the perfusate through a blood vessel can be modeled as laminar flow though a circular pipe. This flow can be calculated from the Hagen–Poiseuille formula:

$$Q_{pipe} = \frac{\Delta P \pi D^4}{128 \mu h} \tag{4.1}$$

where Q_{pipe} is the flow rate through the pipe, ΔP is the pressure difference, D is the diameter of the pipe, μ is the viscosity of the flow, and h is the length of the pipe (blood vessel).

Taking that the area of the vessel to be negligible in comparison to the area of

the sample, the flow rate through the tissue is assumed to be the flow rate through a complete sample (Q_{tissue}). Then the effective flow rate, $Q_{effective}$ is,

$$Q_{effective} = Q_{tissue} + Q_{pipe}. \tag{4.2}$$

The permeability of a tissue sample containing a blood vessel can be calculated from Darcy's law using $Q_{effective}$.

While the smallest capillaries may be as small as 5 μm in diameter, the length of these vessels are short as they are used to connect other larger vessels. Only larger vessels can span the distance across the sclera. With a minimum vessel diameter of $20\mu m$, Q_{pipe} is calculated to be 7.94 x $10^{-6} cm^3/s$, or nine times the expected flow through the scleral tissue sample. The resulting permeability with a 20 μm-diameter blood vessel is then nine times the permeability of a tissue sample without the blood vessel. Thus, any vessels present would be expected to yield large errors in permeability which were not detected in the current study.

Although blood vessels may have been present in the sample, these blood vessels did not significantly alter the scleral permeability.

4.2 Flow Through Other Vessels

Although the tissue sample was selected to minimize blood vessels, other vessels, related to the location of the eye from which the sample was taken, may have been overlooked. In Smith and Johnson's study, the permeability values for the posterior samples were comparable to values from previous studies, although the values for the anterior samples were much higher. Smith and Johnson attributed this discrepancy to vessels found in the anterior chamber such as aqueous veins.

As the aqueous veins are only found in the anterior sclera, this is not a concern in the present experiments, as tissue samples were only taken form the posterior area of the eye.

4.3 Hydration of the Tissue

Tissue hydration and compaction may also affect the permeability. In Hedbys and Mishima's study (1962), they also determined the relationships between tissue thickness and hydration. Hydration is expressed as the weight of water per unit of dry tissue weight. To decrease the hydration in Hedbys and Mishima's study, different amounts of water in the tissue samples of bovine cornea were allowed to evaporate. The relationship between thickness and hydration was found to be linear for both corneal and scleral tissues (Hedbys and Mishima 1962; Fatt and Hedbys 1970a,b).

Changes in permeability with hydration may be explained by the changes of the ECM with hydration. For dehydrated cornea tissue, the fibers remain constant, but the interfibrillar space changes (François et al, 1954; Maurice, 1957). The decrease in thickness with dehydration must then result from changes in the spacing of the amorphous ground substance. Then dehydration results in a higher concentration of the ECM and a lower hydrodynamic permeability of the tissue.

Although hydration does affect permeability, the permeability measurements of this study were not significantly affected by changes in hydration. The tissue samples were constantly covered in saline solution which should not significantly affect the hydration of the tissue.

4.4 Compaction of the Tissue

Compaction may also affect tissue permeability. With Fatt's method for measuring permeability (1968), the tissue sample was flooded with solution (distilled water or unbuffered saline) and allowed to swell to a minimum pressure of 12 mm Hg. Pressure was then added to compressed the tissue and determine the relationship between thickness and swelling pressure. The swelling pressure is in effect the compaction pressure. The swelling pressure was found to be inversely proportional to hydration. Since permeability is directly related to hydration, any compaction should lower a tissue's permeability.

However, as the compression was less than 5 % of the tissue thickness, the effects of compaction on the tissue hydration and hydrodynamic permeability are minimal.

4.5 Conclusions

A direct perfusion apparatus was designed and built for measuring the hydrodynamic permeability of scleral tissue. The design of the experimental perfusion cell and procedure took into account concerns about vessels through the tissue and other factors which would distort the measurements. Using the apparatus, a scleral permeability of 3.643 x $10^{-14} cm^2$ was determined from the experimental data and Darcy's Law. This value is similar to the values, 1.9 x $10^{-14} cm^2$ and 1.6 x $10^{-14} cm^2$, found in earlier studies.From these results the conclusion can be made that the experimental perfusion cell can be used to measure the hydrodynamic permeability of sclera.

However, the perfusion cell can be further improved. The accuracy of the tissue compression can be improved with more accurate machining of the variable spacer rings and with a better selection of spacer rings. For this study, only five spacer rings were made. With better machining skills, the tolerance of the spacer ring thickness can be much smaller than the +/- 0.03 mm.

Another recommendation is the selection of a different part or a different supplier of the porous plates in the perfusion cell. The quality of the fritted disks supplied by Corning Glassware Company was very inconsistent.

With the implementation of the improvements, the perfusion cell can then be used to determine the permeabilities of other connective tissue. Reliable values of hydrodynamic permeability are measureable with the experimental apparatus. This perfusion apparatus will be useful to determine the hydrodynamic permeability in further studies of the relationship between permeability and age-related changes in composition of connective tissue.

Chapter 5

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