Continuous Monitoring of Intraocular Pressure From the Sclera

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Institute of Medical Science
University of Toronto

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Abstract

Glaucoma is an optic neuropathy often associated with increased intraocular pressure (IOP) that can lead to impaired vision and blindness. Frequently asymptomatic, this eye disease may be undetectable until vision is severely compromised. The primary goal of glaucoma management is to prevent progressive nerve damage by maintaining a reduced IOP in order to preserve vision and maintain a patient’s quality of life. IOP is a major risk factor for glaucoma, however, studies have shown that throughout a 24-hour period IOP is not constant and fluctuates throughout the day (Sit, 2009; Liu et al., 2009). This results in the clinical dilemma that diurnal changes in IOP, particularly during sleep, in patients with glaucoma may not be accurately evaluated during the regular practitioners’ office hours when diagnosis and management are provided. Since lowering and stabilizing IOP remains the only proven way to reduce disease progression (Barkana et al., 2006; Singh et al., 2008; Leske et al., 2007; Bengtsson et al., 2007; Caprioli and Coleman, 2008), the development of a continuous IOP monitoring device, for human use, is critical. To date, there remains no commercially available non-invasive human device that can reliably and accurately measure IOP throughout the 24-hour period. There is an experimental contact lens device available in Europe (Leonardi et al., 2009), but there are significant disadvantages using a silicon contact lens on the cornea. The objective of this thesis was to demonstrate the efficacy of a noninvasive strain gauge sensor, placed on the sclera, to detect changes in IOP. The
**specific aims** are to provide proof-of-principle for a novel, scleral mounted device for measuring IOP by: (1) establishing whether IOP can be recorded from the conjunctival and scleral surface using enucleated porcine eyes; (2) establishing whether IOP can be recorded from the conjunctival surface in a live pig model; and (3) to examine the inflammatory response of a hydrogel contact lens placed overnight on the scleral, bulbar conjunctiva as a plausible carrier for the strain gauge sensor. Changes in IOP can be accurately assessed by measuring changes in the radius of curvature of the sclera in enucleated tissue as well as in a live porcine model. Analysis of silicone hydrogel contact lenses, approved for overnight wear on the cornea, showed that they may be safely mounted on the upper bulbar conjunctiva and present a promising carrier material for a 24-hour IOP monitoring device. These proof-of-principle experiments are an essential step to the realization of a safe, scleral mounted, 24-hour IOP recording device that has the potential to significantly impact the way glaucoma is diagnosed and managed.
Acknowledgments

“There must be a beginning of any great matter, but the continuing unto the end it be thoroughly finished yields the true glory.”

-Francis Drake, 1587

There were times during my graduate experience that I thought that I would never finish. But now, as I am writing out my acknowledgements, I am truly pleased to know that it is all coming to an end! My PhD experience has been a unique one. Most definitely defined as a rollercoaster of emotional as well as intellectual drainage. Yet, I would not change any aspect of it. This process, though quite long and tedious, has altered the way I think, listen and learn. I have accepted the challenges and have learned to achieve greatness through the mistakes that have come along the way. Hence, I consider my graduate experience to have been a great one particularly due in part to the contributions of advice, time and encouragement from many people who helped make this PhD project possible. First and foremost, Dr. John Flanagan, without his support and encouragement, this project would of never been possible. I thank him tremendously for taking me on, and believing in my abilities to make this project a success.

I am grateful to Drs. Graham Trope and Chris Hudson for their invaluable contributions as members of my thesis committee. Their dedication to ophthalmology and vision sciences, in particularly to the field of glaucoma and diabetic retinopathy, is eminent. Their desire to help construct this project was instrumental in making this thesis work.

I would like to thank Dr. Carol Westall for her encouragement and support during my PhD work. Her door was always open for me. Carol instilled in me a positivity that allowed me to complete my work. Thanks to Dr. Kostadinka (Dida) Bizheva for her advice throughout this experience and even for wanting to take me on as a student when times were tough. I would like to recognize Mrs. Inka Tertinegg for all her help and involvement in this project. Inka’s comments and helpful advice along the way helped raise the scientific quality of this work. I also would like to thank Dr. Michael Ward, who passed away in November of 2009. A brilliant individual, he was kind and always took the time to listen to me.
Thanks are also due to several researchers and staff at other organizations. I would like to thank the Centre for Contact Lens Research at the School of Optometry, University of Waterloo. In particular Drs. Maud Gorbet, Nancy Keir, Lyndon Jones, Doerte Luensmann, and Kathy Dumbleton for their advice and help in setting up the clinical protocol for this project. Gratitude also goes to Cameron Postinikoff for his help with flow cytometry and for always responding to my emails quickly and efficiently. I also would like to acknowledge Dr. Jeremy Sivak and his lab; the staff of the animal vivarium at the University Health Network, in particular Sandra Lafrance and Trista Murphy, who greatly helped with the live animal experiments.

Most importantly, I am forever indebted to my husband, Nick Stavropoulos, for his understanding, endless patience and encouragement when it was required. For his ever lasting moral support and unyielding love to push me in completing this work. I thank him for making me laugh when times were tough and adding humor to the situation, making me know that no matter what, Aldo would always have a position for me! You’ve taught me to be patient and to realize that there are more important things in life that should be cherished.

I thank my parents and my in-laws for their support throughout this process. Without their help and guidance none of this would of been possible. I also would like to thank my sister and brother for their encouraging thoughts, funny things to say, and always being by my side.

This project would not be possible without the financial support of the Glaucoma Research Society of Canada, the Vision Science Research Program Scholarship and the Peterborough K. M. Hunter Scholarship.
Dedications

I dedicate this thesis to my two beautiful daughters,

Angelina and Georgia.

Without them, life would be insignificant.
Contributions

Aphrodite Stavropoulos (author) solely prepared this thesis. All content of this PhD thesis, including planning, execution, analysis and writing of all original research and publications was prepared in whole or in part by the author. The following contributions by other individuals are formally and inclusively acknowledged:

Dr. John Flanagan (Supervisor and Thesis Committee Member) – Planned, mentored, guided and aided with analysis of experiments as well as thesis preparation

Mrs. Inka Tertinegg – Mentored and aided with design and fabrication of transducer apparatus as well as analysis of experiments for Chapter 2 (Figures 2-4 to 2-10).

Dr. Chris Hudson (Thesis Committee Member) – Guided, mentored and aided with analysis of experiments as well as thesis preparation.

Dr. Graham Trope (Thesis Committee Member) – Guided, mentored and aided with analysis of experiments as well as thesis preparation.

Dr. Maud Gorbet – Guidance in interpretation of results and assistance in planning, executing and analyzing experiments for Chapter 4 (Figure 4-3 and 4-4).
Table of Contents

ACKNOWLEDGMENTS ........................................................................................................ IV

DEDICATIONS .................................................................................................................. VI

CONTRIBUTIONS ............................................................................................................ VII

TABLE OF CONTENTS ..................................................................................................... VIII

LIST OF TABLES ................................................................................................................ VIII

LIST OF FIGURES ............................................................................................................ XI

CHAPTER 1 GLAUCOMA AND INTRAOCULAR PRESSURE ............................................... 1

1 INTRODUCTION ............................................................................................................ 2

1.1 HISTORY OF GLAUCOMA AND INTRAOCULAR PRESSURE .................................. 2

1.2 GLAUCOMA .................................................................................................................. 3

1.2.1 Epidemiology ......................................................................................................... 5

1.2.2 Pathogenesis .......................................................................................................... 5

1.2.3 Diagnosis of Glaucoma .......................................................................................... 6

1.2.4 Managing Glaucoma .............................................................................................. 8

1.2.5 Clinical Dilemma ................................................................................................... 11

1.3 GLAUCOMA AND IOP ............................................................................................... 12

1.3.1 Intraocular Pressure (IOP) .................................................................................... 12

1.3.2 IOP and Diurnal Variations ................................................................................... 16

1.3.3 IOP and Animal Models ......................................................................................... 22

1.3.4 IOP Measuring Techniques ................................................................................... 24

1.3.5 Continuous Measurement of IOP ......................................................................... 30

1.4 STRAIN GAUGE ......................................................................................................... 38

1.4.1 Strain Gauge and Electrical Resistance ................................................................. 38

1.4.2 Thermal Output ...................................................................................................... 39

1.4.3 Pre-Wired Strain Gauge ........................................................................................ 41

1.5 RATIONALE FOR A “SCLERAL” SENSOR ................................................................ 43

1.6 THESIS ORGANIZATION .......................................................................................... 46

1.7 RATIONALE, AIMS AND HYPOTHESIS .................................................................. 47

viii
CHAPTER 2 ASSESSMENT OF PRE-WIRED STRAIN GAUGE ON SCLERAL AND CONJUNCTIVAL TISSUE

2 SUMMARY ........................................................................................................................... 49

2.1 INTRODUCTION ........................................................................................................... 50

2.2 MATERIALS AND METHODS ...................................................................................... 52

2.2.1 Experimental Design and Procedures ...................................................................... 52

2.2.2 Strain Gauge Sensor ................................................................................................. 56

2.2.3 Data Analysis ............................................................................................................ 57

2.3 RESULTS ....................................................................................................................... 57

2.3.1 Preliminary Enucleated Human Eye Results .............................................................. 57

2.3.2 Enucleated Porcine Eye Results ............................................................................... 60

2.3.3 Thermal Drift Experiments ..................................................................................... 64

2.3.4 Calibration Experiments ......................................................................................... 67

2.4 DISCUSSION .................................................................................................................. 82

CHAPTER 3 CONTINUOUS SCLERAL INTRAOCULAR PRESSURE MONITORING IN A LIVE PORCINE MODEL

3 SUMMARY ....................................................................................................................... 87

3.1 INTRODUCTION ........................................................................................................... 88

3.2 METHODS AND MATERIALS ...................................................................................... 90

3.2.1 Animals ..................................................................................................................... 90

3.2.2 Strain Gauge Sensor ............................................................................................... 91

3.3 RESULTS ....................................................................................................................... 94

3.4 DISCUSSION .................................................................................................................. 105

CHAPTER 4 COMPARISON OF THE CELLULAR RESPONSE TO OVERNIGHT CONTACT LENS WEAR ON THE SCLERA AND CORNEA AS AN INITIAL CHARACTERIZATION FOR THE USE OF AN IOP MONITORING DEVICE

4 SUMMARY ....................................................................................................................... 109

4.1 INTRODUCTION .......................................................................................................... 110

4.2 MATERIALS AND METHODS ...................................................................................... 112

4.2.1 Participants and Study Visits .................................................................................. 113

4.2.2 Cell Processing ....................................................................................................... 114

4.2.3 Contact Lens Material ............................................................................................ 116

4.2.4 Statistical Analysis ................................................................................................. 116
CHAPTER 5 CONCLUDING SUMMARY: GENERAL DISCUSSION AND FUTURE DIRECTION - A PARADIGM CHANGE IN GLAUCOMA CARE

5 CONCLUDING SUMMARY .................................................................................................................. 126

5.1 Future Direction ............................................................................................................................ 131

REFERENCES ...................................................................................................................................... 139
List of Tables

Table 1-1. List of Invasive and Non-invasive Continuous IOP Monitoring Devices

Table 2-1. Pre-Wired Strain Gauge Specifications

Table 2-2. Rho Values of Increasing and Decreasing IOP Manipulations in Enucleated Porcine Eyes with the Conjunctiva Removed and with the Conjunctiva Left Intact

Table 2-3. Sensitivity Values of the Strain Gauge When Placed on the Sclera and the Conjunctiva for Increasing and Decreasing IOP

Table 3-1. Rho Values of Increasing and Decreasing IOP Manipulations in a Live Porcine Model

Table 3-2. Sensitivity of Strain Gauge with Increasing and Decreasing IOP in a Live Porcine Model

Table 4-1. Study Procedures and Duration for Overnight Contact Lens Wear on the Bulbar Conjunctiva and the Cornea

Table 4-2. Live PMN and Epithelial Cell Counts with Trypan Blue
List of Figures

Figure 1-1. Differentiation Between Primary Open-Angle Glaucoma and Primary Closed Angle Glaucoma

Figure 1-2. Cupping of the Optic Nerve Head

Figure 1-3. Trabecular Meshwork Outflow Pathway

Figure 1-4. Circadian Variation of IOP During Sleep

Figure 1-5. Fluorescein Pattern Showing a Circular Meniscus Divided Into Superior and Inferior Arcs When Applanated Against the Corneal Surface

Figure 1-6. Illustration of the Scleral Ring Guard

Figure 1-7. Illustration of Contact Lens Corneal IOP Monitoring Device

Figure 1-8. 24-Hour Variations of Central Corneal Thickness, Intraocular Pressure and Corneal Hysteresis in Healthy Young Individuals

Figure 1-9. Dynamics of Bonded Strain Gauge

Figure 1-10. Wheatstone Bridge Configuration

Figure 2-1. Illustration of Enucleated Porcine Eye with Upper and Lower Eyelids Securing Strain Gauge

Figure 2-2. Photographs of Enucleated Porcine Eyes Obtained From a Local Abattoir

Figure 2-3. Schematic of Transducer Set-up

Figure 2-4. Strain Gauge Deformation from IOP Changes in a Human Donor Eye When Placed on the Bulbar Conjunctiva

Figure 2-5. Strain Gauge Deformation from IOP Changes in a Human Donor Eye When Placed Directly on the Sclera

Figure 2-6. Profile of Enucleated Porcine Eye with Strain Gauge Bonded to Sclera with Ethyl-2-Cyanoacrylate

Figure 2-7. Profile of Strain Gauge Displacement Against IOP Alterations of an Enucleated Porcine eye

Figure 2-8. Profile of Strain Gauge Deformation with IOP Change in an Enucleated Porcine Eye
Figure 2-9. Transducer Versus Strain Gauge Displacement (mV)

Figure 2-10. Recordings of Strain Gauge Voltages (mV) Over A Five-Minute Period

Figure 2-11. Thermal Drift of Strain Gauge with No Pressure Induced for Eye5OS

Figure 2-12. Thermal Drift Analysis of Strain Gauge Sensor

Figure 2-13. Calibration of Strain Gauge Sensor with Half-Bridge Circuit

Figure 2-14. Correlation of Transducer to IOP Manipulations

Figure 2-15. Recordings of Strain Gauge Sensor in Response to IOP Changes When Applied to the Sclera in Enucleated Porcine Eyes (N=6)

Figure 2-16. Recordings of Strain Gauge Sensor in Response to IOP Changes When Applied to the Conjunctiva in Enucleated Porcine Eyes (N=8)

Figure 2-17. Correlation of Transducer Output and IOP Manipulations to Tonopen-XL Measurements

Figure 3-1. Photograph of Yorkshire Swine Under Anesthesia with Strain Gauge Sensor Adhered to the Conjunctiva

Figure 3-2. Placement of Strain Gauge Sensor on the Superior, Temporal Region

Figure 3-3. Schematic of Experimental Setup for Live Porcine Model

Figure 3-4. Recordings of Strain Gauge Sensor in Response to IOP Changes When Applied to the Conjunctiva in a Live Porcine Model (N=9)

Figure 3-5. Electrical Interference of Ventilating Machinery on IOP Monitoring Device

Figure 3-6. Recording of Strain Gauge Sensor Under Repetitive Ocular Movement in A Porcine Model Under Anesthesia

Figure 4-1. Diagram of Cell Collection with the Ocular Surface Cell Collection Apparatus (OSCCA)

Figure 4-2. Illustration of the AFUs of PMNs of Various Cell Surface Markers

Figure 4-3. Percentage of Tear Film Neutrophils in the Cell Collection for Baseline
Chapter 1 Glaucoma and Intraocular Pressure
1 Introduction

This review chapter is divided into four sections. The first section (1.2) gives a description of glaucoma and IOP, as well as the clinical challenges associated with measuring IOP. Section 1.3 focuses on IOP during sleep. It should be noted that IOP is a hallmark for the pathogenesis of the glaucomas and that importance of understanding and documenting IOP stems from the notion that it is the only modifiable risk factor that can be manipulated to improve the state of glaucoma. Although experimental and clinical data have provided some understanding of IOP, it is not fully understood as to how the eye responds to IOP at the cellular and molecular levels. This chapter also reviews IOP monitoring devices and the specifications of the pre-wired strain gauge that is used as the primary sensor for the experiments (section 1.4). Finally, section 1.5 discusses the rationale for a “scleral” strain gauge sensor and section 1.6 outlines the aims and hypotheses of the original research for this thesis.

1.1 History of Glaucoma and Intraocular Pressure

Glaucoma is derived from the Greek word *glaukos* (γλαυκός), meaning “opacity of the crystalline lens.” The term glaucoma was first used around 400 B.C. in the Hippocratic aphorisms to describe an ocular disease in elderly people (Fronimopoulos & Lascaratos, 1991). It was identified as a glossy appearance of the pupil and ultimately considered a disease of the lens or cataracts. The distinction between glaucoma and the latter did not occur until the early eighteenth century. In 1818 Dr. Antoine-Pierre Demours, a French ophthalmologist gave the first description of glaucoma that included the notion of a raised ocular tension. In 1823, Dr. G. J. Guthrie identified firmness of the eye as a characteristic of glaucoma. In 1835, Dr. William McKenzie, a Scottish clinician, described an elevation of IOP in both chronic and acute forms of the disease. By the mid-19th century, elevated IOP was accepted as a recognized feature of glaucoma (Mantzioros, 2011). In 1863, von Graefe developed the first instrument to measure IOP. It was made to measure IOP through the eyelid or from the sclera. This led to the development of numerous other indentation tonometers that measured the amount of indentation of the sclera induced by a given force (Stamper, 2011). In 1884, the use of cocaine as a topical ocular anesthetic led to the development of various indentation and applanation devices that could be applied directly to the cornea. By the 20th century, IOP, as a measurable parameter, was contingent on the diagnosis and treatment of glaucoma (Stamper, 2011). Yet, it became
questionable as to why certain individuals with glaucomatous optic neuropathy (GON) had normal IOPs and those with no evidence of GON had elevated IOPs. As a result, the definition of glaucoma drastically changed to encompass not only an elevated IOP but also a neuropathy that causes optic nerve damage and visual field loss. Currently, IOP no longer alone defines glaucoma but remains to be its only modifiable risk factor and a critical measurement for the management of the disease. In order to most effectively manage glaucoma, IOP would ideally be measured safely and accurately throughout the diurnal and nocturnal cycle, rather than providing a momentary snapshot. No commercially available device thus far is able to accomplish this. Furthermore, current methods of IOP measurement are confounded by variations in corneal parameters. My work aims to provide proof-of-concept for a non-invasive continuous IOP monitoring device that I hope will improve our ability to monitor and manage glaucoma.

1.2 Glaucoma

Glaucoma is a group of progressive optic neuropathies that are associated with atrophy of the optic disc leading to an increased deterioration of the visual field (McKinnon et al., 2008; Bell et al., 2010; Weinreb and Khaw, 2004). Usually asymptomatic, peripheral vision is primarily affected and progresses to eventually include central vision (Almasieh et al., 2012). A common pathophysiological feature found in all forms of glaucoma is the degradation of retinal ganglion cells (RGCs), neurons located in the innermost layer of the retina with axons in the optic nerve that transmit visual information from the retina to the brain (Nguyen et al., 2011; Almashiet et al., 2012). Although, the cause and progression of the disease is still not fully understood, it most likely includes several etiologies that all lead to degeneration of RGCs and their axons (Sit and Liu, 2009; Weinreb and Khaw, 2004; Minton et al., 2012). Elevated intraocular pressure has been identified as a leading risk factor that can lead to the initiation and progression of the disease (Sit and Liu, 2009; Almasieh et al., 2012; AGIS, 2000; Manni et al., 2008; Hughes et al., 2003; Singh and Shrivastava, 2009). Other factors that have been identified to increase the risk for the development of glaucoma include increasing age (Hollands et al., 2013), race (Vajaranant et al., 2012), corneal thinness (Almasaieh et al., 2012), as well as an individual’s genetic predisposition (Takamoto et al., 2012). Yet, IOP is the only proven modifiable risk factor that can alter the course of the disease when treated (Weinreb and Khaw, 2004; Kakaday et al.,
2009; Moodie et al., 2010). All current treatments, whether surgical or medical, are aimed at lowering IOP (Kakaday et al., 2009; Hughes et al., 2003; Hollands et al., 2013). Hence, the accurate monitoring of IOP is a crucial element in glaucoma management.

Primary open-angle glaucoma (POAG) and primary angle-closure glaucoma (PACG) are the two most common clinical types of glaucoma. The difference between the two is based on the morphological appearance of the iridocorneal angle at which aqueous drainage takes place. In POAG, the iridocorneal angle appears anatomically open and morphologically normal allowing aqueous humor to have free access to the trabecular meshwork, the drainage apparatus in the anterior chamber angle (Figure 1-1). In PACG, the root of the iris is in apposition to the trabecular meshwork causing reduced access for aqueous humor outflow. POAG and PACG are treatable, as well as all other forms of glaucoma, yet visual loss is irreversible and early detection is crucial in maintaining a patient’s quality of life (Skalicky and Goldberg, 2008; Severn et al., 2008; Labiris et al., 2010; Onakoya et al., 2012).

**Figure 1-1. Differentiation Between Primary Open-Angle Glaucoma and Primary Closed Angle Glaucoma.** (A) Primary open-angle glaucoma (POAG) and (B) primary angle closure glaucoma (PACG). The angle refers to the area between the iris and the cornea where aqueous humor flows outwards via the trabecular meshwork. In POAG the aqueous humor has free access to the trabecular meshwork, the drainage apparatus in the anterior chamber angle. In PACG the root of the iris is in apposition to the trabecular meshwork, and the aqueous humor is blocked. PACG may result from various mechanisms that involve (1) pupilary block, where the aqueous humor accumulates in the posterior chamber resulting in the iris to push forward blocking the anterior chamber angle; (2) root of the iris causing a direct blockage of the anterior chamber angle (plateau iris); and (3) swelling or an increased size of the ciliary body pushing against the root of the iris towards the trabecular meshwork.
1.2.1 Epidemiology

Glaucoma is the second leading cause of blindness worldwide after cataract (Weinreb and Khaw, 2004), occurring in 1-2% of the North American population, drastically increasing over the age of 50 (Friedman et al., 2004). In 2000, the number of people with glaucoma in the world was estimated to be nearly 66.8 million, with 6.7 million suffering from bilateral blindness (Cedrone et al., 2008). By 2010, it was estimated that glaucoma would have caused bilateral blindness in 8.4 million people and 11.2 million people by 2020 (Cedrone et al., 2008). POAG accounts for 90 percent of glaucoma cases in North America and Europe and is the leading cause of blindness in African Americans (Weinreb and Khaw, 2004). PCAG accounts for less than 10 percent of glaucoma cases in North America and Europe but as much as half in Asian countries. It is estimated that 50 percent of individuals with glaucoma have currently undetected disease and that half of those were estimated to have advanced disease (Hollands et al., 2013).

1.2.2 Pathogenesis

Several theories have been proposed to explain the pathogenesis of glaucoma. Although the cause of the disease is still not fully understood, these theories are thought to lead to the initiation of RGC loss (Almasieh et al., 2012). According to the mechanical theory, increased IOP causes stretching of the laminar cribosa, potentially damaging the retinal ganglion cells (RGC) (Girard et al., 2009), and reducing axoplasmic flow along the axons. The associated posterior bowing of the lamina cribosa lends support to this theory. Evidence shows that lowering IOP reduces the risk of development or slows the progression of glaucoma (Barkana et al., 2006; Singh et al., 2008; Leske et al., 2007; Bengtsson et al., 2007; Caprioli and Coleman, 2008). However, elevated IOP alone cannot explain all glaucoma cases, as a substantial proportion of glaucoma patients do not have IOP elevated above the normal range, such as in Normal Tension Glaucoma (NTG). Hence, the vascular theory proposes that glaucoma is a result of insufficient blood flow due to an increase in IOP or other risk factors, reducing ocular blood flow to the supporting tissue. Therefore, additional risk factors are considered critically important. Although these two theories exist, it is generally accepted that they occur concurrently in the development of glaucoma (Sit and Liu, 2009). Once the disease process has commenced, it causes damage and loss of neural tissue, glial cell activation and tissue remodeling, finally leading to formation of optic nerve cupping, the main clinical characteristic of glaucomatous optic neuropathy (GON) (Figure 1-2). Other physiological parameters indicative of optic disc changes in glaucoma are
increased pallor of the nerve head, nasal displacement of central retinal vessels, baring of the lamina cribosa and splinter hemorrhage (Infeld and O’Shea, 1998).

![Image of optic nerve head](image)

**Figure 1-2. Cupping of the Optic Nerve Head.** Early progression can be seen by comparing sequential photographs of the optic nerve. Changes in the inferior rim can be seen when comparing photographs taken 4 years apart. There are subtle changes in the course of the smaller vessels when comparing the inferior optic nerve at baseline (A) to 4 years later (B). There is also thinning of the inferior temporal rim and the development of an associated, wedge-shaped, nerve fibre layer defect.

### 1.2.3 Diagnosis of Glaucoma

The diagnosis of glaucoma includes the evaluation of three primary parameters, structure of the optic disc and nerve fibre layer, function, by assessment of the visual field (perimetry) and IOP measurement (Tuck and Crick, 1997; Downs et al., 2009). Determination of the presence of optic disc neuropathy, visual field abnormalities and elevated IOP are all required to make a positive diagnosis of glaucomatous optic neuropathy (GON). A diagnosis cannot be made on elevated IOP alone, for patients that present without any indication of optic nerve head or nerve fibre layer damage, or visual field loss, but with elevated IOP are regarded as having ocular hypertension (Kwon and Caprioli, 2006).

Determination of optic disc size is extremely important in diagnosing glaucoma. The size of the optic disc is associated with the size of the optic cup and the neuroretinal rim (Hoffmann et al.,
The neuroretinal rim is the location of the bulk of the axons of the retinal ganglion cells and is the tissue between the cup and the disc margin. In normal patients, the neuroretinal rim has an orange-red hue due to the capillary bed, loss in color is apparent in glaucoma (Uhler, 2003). A normal optic disc is usually vertically oval. The optic disc contains a central physiological cup that is horizontally oval in shape and lacks color due to the absence of optic nerve fibers. The detection of characteristic glaucomatous optic disk atrophy involves the measurement of the size and shape of the optic disc and neuroretinal rim. A ratio of the optic cup diameter to the optic disc diameter also known as the cup to disc ratio (CDR) is used as a measurement to assess the progression of glaucoma. When the term “cupping” is used it specifically refers to the loss of nerve fiber axons at the level of the lamina cribosa that begin at the inferior and superior margins of the optic cup. Several physiological changes of the optic disc are attributable to optic nerve cupping. These include but are not limited to a documented increase in diameter or depth of the cup; a CDR of greater than 0.5; a CDR difference of more than 0.2 between two eyes; extension of the cup to disk margin; notching of the cup; exposed lamina cribosa; and peripapillary atrophy. If glaucomatous atrophy is not treated this will result in the loss of all neuroretinal rim tissue. Further, disk size within a population and among a population may also vary and that these variations may influence the susceptibility to glaucoma or the likelihood that a diagnosis of glaucoma may be made (Hoffmann et al., 2007).

Different measuring techniques are used to evaluate the progression of disc changes in glaucoma and for estimating the optic disc size. Slit-lamp biomicroscopy, planimetry, confocal scanning laser ophthalmoscopy and optical coherence tomography are a few of the measurement techniques available. Yet, some of the measurement techniques are more applicable to clinical practice than others. The respective strengths and limitations of these measuring techniques will not be discussed. What should be noted is that the assessment of the optic disc and nerve fibre layer is an important parameter in the diagnostic evaluation for glaucoma but needs to be in association with visual field examination and IOP measurement to be termed as glaucomatous optic neuropathy.

Visual field testing examines visual function in the peripheral and central fields of vision and is useful in documenting the progression of glaucoma. Visual field loss in glaucoma is often proportionate to the degree of glaucomatous defects (Crabb, 2009). In the early stages of glaucoma, peripheral changes in visual fields may be observed (Pan and Varma, 2011). Central
vision is primarily preserved during the early stages of the disease although sometimes defects may involve fixation (Pan and Varma, 2011). Typical visual field loss is directed by the organization of the retinal nerve fiber layers as they enter into the optic disc, with fibers from the temporal retina being most susceptible to damage effecting the superior hemifield (Crabb, 2009). The fibers from the fovea centralis of the central retina are less affected by increased pressure.

The measurement of IOP is used as a diagnostic outcome in association with optic nerve neuropathy and visual field abnormality. The normal range of IOP (mean ± SD) is 15.5 (± 2.57) mmHg. Historically this was treated as a Gaussian distribution whereby two standard deviations include the values of 95 percent of the population, and an IOP of >21 mmHg may be considered outside of normal limits, i.e. ocular hypertensive. In reality the distribution is skewed and a cut off of 21mmHg overestimates abnormality. Yet, when defining glaucoma, there is no safe level of IOP depending on other parameters. For instance, some patients may develop optic neuropathy with low levels of IOP while others may not develop any forms of optic nerve neuropathy with high levels of IOP (Infeld and O’Shea, 1998). Again, elevated IOP is an important risk factor because it is the only modifiable risk factor when considering management the disease. In relation to POAG, 80 percent of individuals will exhibit an elevation in IOP, referred to as high-tension glaucoma. This should not be mistaken with ocular hypertension that is clinically defined as having an IOP greater than 21 mmHg where the appearance of the optic disc and the visual fields are within normal limits.

1.2.4 Managing Glaucoma

Current standards for managing glaucoma include pharmacotherapy or surgery aimed at lowering IOP or preventing a rise in IOP to minimize cell death (Gulati et al., 2011; Heijl et al., 2002; AGIS 2000). Lowering IOP by medical or surgical means remains the only proven way to slow progression of glaucomatous optic nerve neuropathy (Gulati et al., 2011). Even at advanced stages of glaucoma, lowering IOP has the strongest indication of protecting the optic nerve and remaining visual fields (Gessesse and Damji, 2013). Therapeutic agents lower IOP by decreasing aqueous humor inflow (i.e. carbonic anhydrase inhibitors and β-blockers), enhancing the trabecular outflow of aqueous humor (i.e. cholinergic agents and prostaglandin analogues) or a combination of these mechanisms (α₂-adrenergic agonists) (Gulati et al., 2011; McGinnon et al., 2008). Topical administration of drugs is often considered as initial therapy for glaucoma. Their
mechanisms target the reduction of aqueous humor production in the ciliary body or an increase in outflow through the uveoscleral pathways, thus altering the variables of aqueous humor dynamics (Gulati et al., 2011).

Aqueous humor production decreases slowly with age and varies with the circadian cycle (McLaren, 2009). During night aqueous humor production decreases, which renders certain drugs non-functional during that time period. The efficacy of IOP lowering medications are usually elevated during the diurnal period, but whether the same is true during the nocturnal time frame is still unclear (Liu et al., 2004). Hence, the management of glaucoma via pharmacotherapy will improve with the development of a continuous monitoring IOP device that can measure IOP throughout a 24-hour period, particularly night under closed eyelid conditions. This will provide further information on the efficacy of commonly used glaucoma drugs that have been shown to be ineffective during sleep. For instance, β-blockers have exhibited an inability to lower IOP during sleep conditions (Orzalesi et al., 2000; Liu et al., 2009). In contrast, prostaglandin analogues, lower IOP by increasing aqueous humor outflow, have shown a sustained lowering effect of IOP during the nocturnal period (Racz et al., 1996). A study by Liu and colleagues (2004) prospectively compared the nocturnal effects of once daily timolol (β-adrenergic antagonist) and latanoprost (prostaglandin analogue) in patients with hypertension and glaucoma. Eighteen patients with early glaucomatous changes or ocular hypertension (aged ranged from 41 to 79 years old) were topically administered treatments with timolol (0.5% Timoptic-XE), latanoprost (0.005% Xalatan), and medication for a four-week period. Topical drops of timolol were given one in the morning upon awakening and topical drops of latanoprost were given once in the evening at bedtime. After each treatment period, patients were enrolled in a sleep laboratory were measurements of IOP were recorded with a pneumatonometer every two hours. IOP measurements were performed in the sitting and supine position during the diurnal period and only in the supine position during the nocturnal period. The study demonstrated that although both medications were effective in lowering IOP during the diurnal period, only latanoprost lowered IOP during the nocturnal period (Liu et al., 2004). Surgical procedures seek to lower IOP by increasing aqueous outflow or decreasing aqueous humor production as a means to manage glaucoma. The most common surgical procedure for the management of glaucoma is a trabeculectomy. In trabeculectomy, an alternative drainage site for aqueous humor is formed by creation of a fistula between the anterior chamber and the
subconjunctival space via a subscleral excision of a part of the trabecular meshwork. This surgical procedure bypasses outflow resistance by shunting aqueous humor through or around the trabecular meshwork. A trabeculectomy is considered a safe surgical procedure with the expectation of approximately a 30 percent reduction in IOP within a six-month postoperative time period (Maeda et al., 2013; Minckler et al., 2005).

Another surgical procedure for the management of glaucoma is a canaloplasty. The canaloplasty involves the insertion of a microcatheter into Schlemm’s canal to facilitate aqueous humor outflow. Glaucoma drainage implants and glaucoma shunts have also been employed as surgical means to lower IOP by penetrating the trabecular meshwork and cannulating Schlemm’s canal or creating a path through the scleral wall. Laser treatments such as cyclophotocoagulation laser aim to diminish the ciliary body as a means of decreasing aqueous humor production.

1.2.4.1 Socio-economic Costs of Managing Glaucoma

Glaucoma is considered one of the five major causes of vision loss in North America, along with age-related macular degeneration, diabetic retinopathy, cataract and refractive error (Cruess et al., 2011). The economic costs of vision loss attributed to glaucoma is rapidly increasing as society ages. Older adults with loss of vision are twice as likely as those with normal vision to report difficulty with daily living tasks such as preparing meals, getting in and out of bed, going outdoors and managing medications (Cardarelli and Smith, 2013). The financial costs of vision loss in Canada in 2007 was estimated to be $15.8 billion per year with $8.6 billion representing health system expenditure and $4.4 billion productivity loss due to unemployment and premature death (Cruess et al., 2011). This amounts to a financial cost of $19,370 per person with vision loss per year in Canada. The financial cost is expected to increase as the baby boomer generation continues to age. In the United States of America (USA), the total direct and indirect costs of adult visual impairment are currently $51.4 billion. While new surgical and medicinal therapies have been introduced to manage glaucoma, a paradigm shift in management is needed to outway the substantial costs (Cardarelli and Smith, 2013).

Lee et al. (2006) conducted a retrospective study of medical records of 151 individuals and found that average cost of glaucoma therapy increased from $623 per patient per year with early-stage glaucoma to $2511 per patient per year with vision loss attributed to glaucoma. The trend of
rising costs associated with glaucomatous vision loss in North America will have a tremendous impact on patients and society as a whole (Rein et al., 2006).

1.2.5 Clinical Dilemma

The primary goals of glaucoma management are to preserve visual function and maintain a patient’s quality of life (Heijl et al., 2012). All therapies currently used for the treatment of glaucoma are aimed at lowering IOP or preventing a rise in IOP in order to minimize progression of neuropathy. Yet, IOP varies throughout diurnal and nocturnal periods. By understanding the circadian rhythm of IOP we can further establish the clinical dilemma that exists when diagnosing and managing glaucoma: diurnal changes in IOP, particularly during sleep, in patients with glaucoma are not easily evaluated. Since reducing IOP remains the only proven way to slow disease progression, establishing an IOP monitoring device that could measure throughout the diurnal and nocturnal cycle would be helpful in the management of patients with glaucoma. Continuous monitoring of IOP is potentially a key component to early diagnosis and treatment. Current methods of IOP measurement are non-continuous and are confounded by variations in corneal thickness and corneal hysteresis. (Kakaday et al., 2009).
1.3 Glaucoma and IOP

Elevated IOP is a major risk factor for the pathogenesis of glaucoma. Various physiological and environmental conditions can influence IOP in the diurnal and nocturnal periods (David et al., 1992; Sit, 2009). These include time of day, respiration, exercise and even method of measurement. If normality is assumed, normal population values of IOP have a mean (SD) of 15.5 ± 2.6 mmHg (Bonomi et al., 1998; Davanger et al., 1991).

Elevated IOP has unquestionably been linked to the development and progression of glaucoma (Hughes et al., 2003; Weinreb, 2005; Singh and Shrivastava, 2009; Medeiros et al., 2007) and lowering IOP remains the only proven way to stabilize its progression (Barkana et al., 2006; Crawford Downs et al., 2011). Despite all that we know about IOP and its relationship to glaucoma there remain many unanswered questions due to our inability to accurately assess IOP over long periods of time and throughout the diurnal cycle, particularly during sleep. Understanding IOP and its variability is essential for the appropriate diagnosis and management of patients with glaucoma, but there is also a need to measure IOP under normal physiological conditions. The next section provides a literature review of the current state of knowledge on IOP, including diurnal fluctuations, nocturnal variations, IOP fluctuations in animals, IOP measuring techniques and continuous IOP measuring devices.

1.3.1 Intraocular Pressure (IOP)

IOP is physiologically determined by the rate of aqueous production in the ciliary body, resistance to outflow through the trabecular meshwork and Schlemm’s canal, resistance to outflow through the uveoscleral tract and episcleral venous pressure (Newell, 1986; Rhee, 2003).

\[ P = \frac{F}{C} + P_e \]

- \( P \) = intraocular pressure
- \( F \) = rate of aqueous humor production
- \( C \) = facility of outflow
- \( P_e \) = episcleral venous pressure
The Goldmann equation where \( P \) represents the IOP, \( F \) the rate of aqueous production and \( C \) is the facility of outflow, summarizes this relationship (Rhee, 2003). Therefore, IOP is the result of a dynamic equilibrium that exists between the aqueous humor formation and the outflow. Aqueous is produced by the ciliary processes (c. 2\( \mu \)l/min) in the posterior chamber and is considered to have a hydrostatic component that is formed by passive leakage of fluid from the blood, and a secretory component that occurs as a consequence of the active transport of ions by the ciliary epithelium (Kniestedt et al., 2008). Aqueous humor circulates throughout the anterior chamber and the majority exits via the trabecular meshwork into Schlemm’s canal, the collector channels and the episcleral veins. Alternative pathways exist including via the uveoscleral pathway but are responsible for relatively small volumes of aqueous humor outflow (Kniestedt et al., 2008). The most common explanation for an increase in IOP is that it results from an increase in trabecular outflow (Weinreb, 2000).

1.3.1.1 Aqueous Humor Dynamics

The production of aqueous humor and the regulation of its outflow are vital physiological processes that are required for proper functioning of the ocular structures. Aqueous humor is a clear fluid that aids in the formation of the anterior and posterior chambers. In a normal eye the flow of aqueous humor against outflow resistance generates an IOP of about 15 mmHg, which is necessary to maintain the normal shape of the eye (Goel et al., 2010). The average volume of the adult human eye globe is approximately 6.5 cm\(^3\) with average globe dimensions of approximately 24 mm from the anterior segment to the posterior segment (Sherman et al., 2006). Aqueous humor comprises about 20 percent of the globe volume and the vitreous body composing the remainder of the volume (Sherman et al., 2006). The impairment of aqueous humor outflow results in an increase in the IOP, which is the central principle of glaucoma treatment (Goel et al., 2010). The aqueous humor plays a vital role in nourishing avascular structures of the eye such as the lens and cornea by providing nutrition, removing excretory products from metabolism and stabilizes the ocular structure (Millar et al., 2006). Aqueous humor also aids in the circulation of mediators and cytokines in the eye during inflammatory conditions as well as various therapeutic agents that are injected into the anterior chamber. The regulation of IOP is maintained by a homeostatic balance that exists between the production and exit of aqueous humor. Aqueous humor is produced by the ciliary body and secreted by the ciliary body epithelial lining into the posterior chamber (aqueous humor inflow). The aqueous...
humor then circulates into the anterior chamber through the pupil and exits via the trabecular meshwork and into Schlemm’s canal (trabecular outflow) and through the peripheral base of the iris into the ciliary body and through the sclera (uveoscleral outflow) (Figure 1-3).

![Figure 1-3. Trabecular Meshwork Outflow Pathway.](image)

**Figure 1-3. Trabecular Meshwork Outflow Pathway.** Schematic diagram illustrating the trabecular meshwork conventional outflow pathway as well as the uveoscleral unconventional outflow pathway. The aqueous humor is formed in the ciliary processes and is secreted to the posterior chamber where it flows to the anterior chamber. Aqueous humor exists via the trabecular meshwork or the uveoscleral pathway. The ciliary tone establishes a balance between the two pathways. (Llobet et al., 2003; © 2013 The American Physiological Society).

### 1.3.1.2 Aqueous Humor Formation

The formation and composition of aqueous humor involves three mechanisms: active secretion, diffusion and ultrafiltration (Goel et al., 2010). Active secretion is responsible for the majority of total aqueous humor formation and is performed by the ciliary epithelium by active transport across a concentration gradient in the blood aqueous barrier. Active secretion requires energy to transport the fluid that is created by the hydrolysis of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). This process is activated by sodium and potassium channels controlled by Na+-K+-ATPase enzyme located in both non-pigmented and pigmented ciliary epithelia (Millar et al., 2006; Caprioli, 1992; Goel et al., 2010). Diffusion and ultrafiltration are passive
physiological processes that require no energy driven mechanisms. Diffusion of solutes cross the lipid properties of the biological membrane between the capillaries and the posterior chamber. Ultrafiltration is the flow of water as a result of hydrostatic pressure, across the fenestrated ciliary capillary endothelia into the ciliary stroma (Goel et al., 2010). Both diffusion and ultrafiltration result in the manifestation of plasma ultrafiltrate in the stroma that formulate the posterior chamber aqueous humor (Goel et al., 2010).

The rate of aqueous humor in a normal human eye, aged 20-83 years old, is approximately $2.4 \pm 0.6 \mu l/min$ (mean ± SD) (Goel et al., 2010). Aqueous humor formation has been known to decline with age, during sleep and in certain systemic diseases such as diabetes (Brubaker, 1991). There is also evidence of a circadian rhythm of aqueous humor flow that follows diurnal variations. Aqueous humor flow seems to be higher in the morning than at night, especially during sleep (Reiss et al., 1984). Normally about $3.0 \mu l/min$ in the morning, aqueous humor flow rate declines to half of that during sleep (Brubaker, 1981). Although aqueous humor flow rate varies in a 24-hour period, different postures, such as supine or sitting, do not affect its outflow mechanism as would for IOP (Carlson et al., 1987).

1.3.1.3 Aqueous Humor Drainage

As mentioned previously, aqueous humor outflow is via both a conventional (trabecular) and unconventional (uveoscleral) pathway. The conventional pathway follows a passive pressure-dependent trans-cellular mechanism as fluid is moved from the trabecular meshwork into Schlemm’s canal and through the inner wall of Schlemm’s canal. Fluid flow through the inner wall endothelium of Schlemm’s canal is controlled by paracellular routes that contain giant vacuoles and pores (Inomata and Tawara, 1984). Elevation in IOP results in structural changes of the endothelium lining Schlemm’s canal that results in an increase in the amount of vacuoles and pores found (Goel et al., 2010). After the aqueous humor exits Schlemm’s canal it enters the aqueous veins where the pressure is 8-10 mmHg and the resistance of the conventional drainage tissues are approximately 3-4 mmHg/µl/min (Phelps and Armaly, 1978). This generates a mean IOP of $15.5 \pm 2.6$ mmHg (mean ± SD) for a normal, healthy eye (Bonomi et al., 1998; Davanger et al., 1991, Goel et al., 2010).
Alternatively, because there is no epithelial or endothelial lining separating the spaces between the trabecular lamellae from the ciliary muscle, aqueous humor may pass to the ciliary body into the suprachoroidal space, through the sclera and into the orbit. This is termed the unconventional or uveoscleral pathway and accounts for 10 percent of total outflow (Millar et al., 2006; Kaufman et al., 1999).

1.3.2 IOP and Diurnal Variations

For consistency this section’s review will employ the following terminology and definitions:

**Diurnal variations:** IOP variations throughout the 24-hour period (Hughes et al., 2003).

**IOP fluctuations:** Short-term changes that occur in IOP from visit to visit and may occur during a 24-hour period.

**IOP peak:** Highest IOP recorded during a specific time period.

**IOP trough:** Lowest IOP recorded during a specific time period.

**Nocturnal IOP:** IOP measurements taken during sleeping hours under darkened conditions in the supine position.

**Target IOP:** Upper limit of the range of measured IOP required in an attempt to stop progressive pressure-induced optic nerve head insult (Anand, 2009).

The glaucoma landmark clinical trials are large scale, prospective, masked, multicentre, randomized studies and include the Ocular Hypertension Study (OHTS) (Kass et al., 2002), Early Manifest Glaucoma Study (EMGT) (Heijl et al., 2002), Collaborative Initial Glaucoma Treatment Study (CIGTS) (Lichter et al., 2001), Collaborative Normal Tension Glaucoma Study (CNTGS) (Collaborative Normal-Tension Glaucoma Study Group, 1998), Advanced Glaucoma Intervention Study (AGIS) (AGIS Investigators, 2000) and the European Glaucoma Prevention Study (EGPS) (Miglior et al., 2002). The OHTS was conducted on patients who presented with a “normal” ocular examination but with elevated IOP between 24 mmHg to 32 mmHg in one eye
and between 21 mmHg and 32 mmHg in the bilateral eye. The aim of the study was to achieve a 20 percent reduction in IOP. The OHTS demonstrated that the risk of progression to glaucoma was significantly reduced in treated patients compared to untreated and that the relative risk of progression increased 10 percent for every increase in mmHg of mean IOP. IOP, age, vertical and horizontal cup-to-disc ratio, and central corneal thickness were identified as good predictors for the progression to glaucoma, with the strongest association being central corneal thickness. Yet, there was a significant inverse correlation between central corneal thickness and lowering IOP, whereas patients who had thicker corneas had a lower measured IOP in comparison to those with thinner corneas (Brandt et al., 2004). In the EMGT, patients with untreated glaucoma and newly diagnosed glaucoma were studied. Groups were randomized to a treatment group that received argon laser trabeculoplasty and/or topical beta-receptor blocker, and a control group that received no treatment. The EMGT study demonstrated that each mmHg of higher mean IOP at follow-up increased the risk of glaucoma progression by 13 percent. A 25% decrease in IOP from baseline reduced the risk of progression by 50 percent (Heijl et al., 2002; Leske et al., 2003). The CIGTS studied newly diagnosed patients with glaucoma who were randomized into a medical therapy and trabeculectomy group with target IOPs determined for each patient. Patients in the trabeculectomy group had a mean IOP of 14 mmHg to 15 mmHg whereas patients in the medical therapy group had a mean IOP of 17 mmHg to 18 mmHg. The rate of visual field loss did not significantly differ between groups (Lichter et al., 2001). The CNTGS compared treatment versus no treatment in patients who presented with an IOP less than 20 mmHg. Target IOPs were set to achieve a 30 percent reduction from baseline IOP. Trabeculectomy was performed in 50 percent of the eyes to achieve the latter target. The CNTGS was the first study to demonstrate that a 30 percent reduction in normal tension glaucoma was beneficial (Anderson et al., 2003). The AGIS examined patients who presented with higher initial IOPs that could not be managed by medication alone. The target IOP was set to less than 18 mmHg and progression was detected by visual field analysis. Relevant findings from this study demonstrated that there was a relationship between IOP lowering and preservation of visual fields. Nevertheless, lowering IOP was independent of baseline IOP, sex, race and systemic disease (Nouri-Mahdavi et al., 2004). The EGPS studied patients who were administered a topical carbonic anhydrase inhibitor (dorzolamide) or placebo without a set target IOP. There was a 15 percent reduction after six months in the treated dorzolamide group and a nine percent reduction after six months in the placebo group. The EGPS study parameters were criticized due to a significant loss in
follow-up for both study groups. The baseline IOP was calculated only from two to three measurements per eye at the screening visit and one measurement per eye at the six month visit. Therefore, the baseline IOP may not have truly been a peak IOP, providing a false representation mean IOP (Quigley, 2005). In summary, results from these large prospective, randomized studies have provided key findings that have linked IOP and glaucoma, showing that lowering IOP decreases progression across all stages of the disease (Wishart, 2009).

Current standards for glaucoma care usually involve single time point IOP readings at each visit, due to time, cost and difficulty. Yet, a series of single time point IOP measurements do not reflect the variations of IOP during a 24-hour period. There are short-term fluctuations and long-term variations of IOP. Short-term fluctuations of IOP occur over hours or days. Twenty-four hour variations in IOP can be categorized as diurnal (daytime), nocturnal (nighttime), and circadian (24-hour). Short-term IOP fluctuations are attributed to changes in aqueous flow dynamics, episcleral venous pressure and trabecular outflow. The pattern of diurnal and circadian short-term fluctuations of IOP vary between individuals with glaucoma and those without. Drance reported the mean range of IOP circadian fluctuation in normal eyes to be 3.7 mmHg with a maximum range of 10 mmHg (Drance, 1960) whereas that in untreated glaucomatous eyes to be 7.5 mmHg with a maximum range of 16 mmHg (Kotecha et al., 2009). IOP tended to be at its highest in the early morning and gradually decrease throughout the day. IOP monitoring between 8:00 AM and 4:00 PM has a 60 % chance of recording the peak IOP. On average, normal eyes are to have low IOP in the sitting position and the highest IOP at night in the supine position, with a 70 % chance of capturing the peak IOP between those same hours. (Kotecha et al., 2009).

Several diurnal IOP studies have reported low IOP recordings during daytime clinic hours and high IOP recordings at night (Liu et al., 1998; Liu et al., 1999; Liu et al., 2003). It is evident that IOP exhibits a diurnal fluctuation, but variations also arise dependent on body position such as sitting or supine, and supine is most often associated with sleep. Recent studies performed in a sleep laboratory demonstrated that IOP is higher in a supine position during the nocturnal sleep period than in a sitting position during office hours in most untreated glaucoma patients (Liu et al., 2003; Mosaed et al., 2005; Buys et al., 2010) (Figure 1-4). Hara et al. (2006)
Figure 1-4. Circadian Variation of IOP During Sleep. IOP exhibits a circadian variation with higher levels during sleep in both newly diagnosed early glaucomatous changes (n=24) (filled symbols) and in normal age matched controls with healthy (n=24) (open symbols); measurements were taken in the sitting (circles) and in the supine (triangles) position. Normal (circle) and glaucomatous (triangle) individuals (Liu et al., 2003; ©ARVO).

also demonstrated that IOP increases in the supine position compared with the sitting position in Japanese glaucoma patients. For each patient, sitting and supine IOP data were recorded for awake and sleep periods. When they developed a composite 24-hour IOP curve that reflected their habitual body positions, the average IOP peaked during the nocturnal/sleep periods. They also recognized that there were fewer IOP peaks during the nocturnal/sleep period when measured in the sitting position only (Hara et al., 2006). In addition, Weinreb and Liu (2006) noted that one of the reasons for the elevation in IOP in the supine position is the increase in episcleral venous pressure. Their study examined 148 patients with untreated glaucoma and measured IOP by noncontact tonometry every two hours from 6 am to midnight and every three hours from midnight to 6 am with patients sitting only and supine only (Weinreb & Liu, 2006). IOP in humans exhibited a repeated diurnal pattern that varies with states of alertness and sleep.
It has been widely documented that the IOP rhythm in glaucoma patients is not accurately represented during regular physician’s office hours (Nakakura et al., 2007). Hughes et al. (2003) documented 24-hour IOP profiles for 29 glaucoma patients and found that peak IOP occurred outside of office hours 51.7% of the time and that high IOP in glaucoma patients often cannot be identified during office hours or even during the entire awake period. Twenty-four-hour monitoring of IOP led to a change of glaucoma management in 79.3% of patients. In addition, Barkana et al. (2006) conducted a study to determine whether IOP monitoring outside of normal office hours added clinically useful information. The study determined that 24-hour monitoring of IOP may disclose a greater role for pressure-related risk for glaucoma progression than previously thought, altering treatment regimes (Barkana et al., 2006). These studies confirm previous observations and help to reconcile differences observed with earlier studies in which only sitting IOP was obtained (Kitazawa & Horie, 1975; Konstas et al., 1997). Understanding IOP during the nocturnal period will help define the relationship between IOP and the progression of glaucoma.

More importantly, since it has been postulated that while sleeping in the supine position, IOP elevates due to episcleral venous flow, knowing the pathophysiology of ocular perfusion pressure, orbital blood pressure and body position will aid in understanding the rhythm of nocturnal ocular physiology. There is a known physiological decrease in systemic blood pressure during sleep. This causes problems for those who are hypertensive because their hypotensive pharmacotherapy may induce a secondary reduction of ocular perfusion in the vessels of the eye, causing chronic ischemia of the optic nerve (Krasinska et al., 2012). Clearly it is difficult to conduct nocturnal studies of IOP since they require sleep laboratories and the inconvenience of being woken up periodically during the night for IOP measurements. However, knowing when IOP peaks and the range of variation would allow for a better understanding of IOP and its involvement in the pathogenesis of glaucoma and may permit better treatment regimens by characterizing an individual’s therapeutic response to IOP lowering.

The debate still remains whether IOP variations and or fluctuations affect the progression of the disease as measured by visual function and optic nerve structure. Since IOP varies, it is hard to obtain an accurate picture over time when only one or two measurements are taken. It has been suggested that short-term fluctuations is a risk factor for glaucoma. Bergea et al. (1999) conducted a prospective study of risk factors in glaucoma and found that both mean IOP and IOP
fluctuations were related with a decrease in visual fields during a two-year time span. They performed diurnal IOP measurements and automated visual field tests on 82 patients, the majority of whom had exfoliative glaucoma, a disease known to be associated with higher IOP fluctuations and variations (Bergea et al., 1999). Both mean IOP and variation were found to be independent predictors of glaucoma progression. In another study, Arsani et al. (2007) used home tonometry to obtain multiple IOP readings at different time points over a five-day period. The study showed a strong correlation between IOP fluctuation and visual field progression (Arsani et al., 2007). Arsani et al. (2007) found that the diurnal variation in IOP, but not the mean IOP, correlated with subsequent progression. Furthermore, Nouri-Mahdavi et al. (2004) investigated the risk factors associated with visual field progression in the Advanced Glaucoma Intervention Study (AGIS). Nouri-Mahdavi reported that long-term IOP fluctuations remained a strong predictor of visual field loss regardless of the inclusion of mean IOP and number of ocular surgeries a patient received (Nouri-Mahdavi et al., 2004). Thus, an analysis of progression in the AGIS by Nouri-Mahdavi et al. (2004) found that visit-to-visit IOP fluctuations and not the mean pressure correlated with progression of disease. On the contrary, the EMGT conducted by Bengtsson et al. (2007) reported that although the mean IOP during follow-up was significantly correlated with risk of progression, the fluctuation of IOP from visit-to-visit over an average of eight years’ follow-up had absolutely no independent effect upon progression. They also found the difference in studies to be based on the fact that they used the mean IOP and visit-to-visit fluctuation of IOP only up to the point of progression and not afterwards, because treatment was likely to have been intensified, and the resultant further lowering of pressure would appear as greater fluctuation (Bengtsson et al., 2007). Whether or not IOP fluctuations and or variations are important in terms of disease progression will continue to be debated until further studies and the ability to accurately monitor IOP over extended periods of time, particularly sleep, can be conducted. Continuous IOP measurements need to be accurately assessed through the diurnal, including nocturnal, periods to provide clinicians with a complete IOP profile to answer the numerous concerns and questions that have not been answered in the latter studies regarding IOP fluctuations and variations and their association with disease management.
1.3.3 IOP and Animal Models

Studies have demonstrated 24-hour IOP rhythms in non-human primates, rats, rabbits, and cats. Circadian rhythms are initiated by internal clocks that do not require environmental timing cues (Lee et al., 1995). Yet, temporal cues from the environment are employed to establish the stage of circadian rhythms and alter their periods to 24 hours (Lee et al., 1995). The most common temporal cue is the light-dark cycle. The suprachiasmatic nuclei neurons contain an internal pacemaker that creates an endogenous rhythm of electrical activity (DelSole et al., 2007), where changes can occur even in the absence of external stimuli. Light reaching the retina transmits information to the suprachiasmatic nuclei through the retino-hypothalamic tract affecting several rhythms including body temperature, hormone levels and activity (DelSole et al., 2007).

In animals, IOP exhibits a circadian component controlled by an endogenous pacemaker in the suprachiasmatic nuclei, that is a paired nucleus, situated above the optic chiasm on each side of the third ventricle (Moore et al., 1996). IOP rhythm in animals has been described to be coordinated by environmental light extending in periods of darkness. This rhythmic regulation that controls IOP in animals has been reported to include the suprachiasmatic nucleus, which regulate the actions of the sympathetic and parasympathetic ocular innervations and also responsible for maintaining the production and outflow of aqueous humor (Chiquet & Denis, 2004).

To define the characteristics of diurnal IOP in rats, Moore et al. (1995) placed ten male Brown Norway rats in a 12-hour light schedule regime followed by a 12-hour dark schedule regime over a 72-hour period. IOP measurements were performed with a Tono-Pen XL tonometer at 4-hour intervals while the animals were awake. Moore et al. (1995) demonstrated that IOP measurements were lower during the 12-hour lighting period (19.3±1.9 mm Hg) in comparison to the 12-hour dark period (31.3±1.3 mm Hg). The authors reasoned that since rats are nocturnal animals an increase in IOP in comparison to light hours is a behavioral state and not a physiological one. Yet, it was also noted that IOP increased before the light cycle change, indicative of a biological rhythm. Another explanation as to why IOP is higher during the dark phase in rats in comparison to the light phase could be due to the fact that there is an increase in aqueous humor, controlled by sympathetic innervation (Chiquet & Denis, 2004; Moore et al., 1995).
Increased IOP at night and circadian rhythm has also been extensively described in rabbits (Rowland et al., 1981; Gregory et al., 1985; Kiuchi & Gregory, 1992; Liu and Dacus, 1991; Liu, 1998). For other animals, no circadian IOP rhythm has been reported as yet (Liu, 1998). Anjou et al. (1961) were the first to describe circadian IOP rhythm in rabbits that were housed under natural light conditions. IOP was found to be high at night and low during the daytime. Liu et al. (1991) investigated the adrenergic mechanisms involved in this IOP increase during dark light phases using selective adrenergic agents. Male New Zealand albino rabbits and male mixed breed pigmented rabbits were housed in a daily 12 hour light and 12 hour dark environment and topically administered an alpha-1-adrenergic antagonist which significantly reduced the circadian IOP elevation (Liu et al., 1991). The authors hypothesized that alpha-1-andrenergic antagonism most likely blocked the signaling pathway of the adrenergic transmitter for an increase in IOP, modifying the circadian rhythm. This finding suggested that an increase in outflow resistance and increase in aqueous flow might contribute to the circadian IOP elevation (Liu et al., 1991). Moreover, many circadian rhythms are dampened by constant light (Lee et al., 1995; Rowland et al., 1981). Rowland et al. (1981) showed that after exposing rabbits to constant light for four days the circadian rhythm of IOP was abolished and IOP remained constant between the daytime low and nocturnal high. Lee et al. (1995) studied whether the loss of circadian rhythm in rabbits in constant light is gradual or rapid. The study hypothesized that a constant light would gradually blunt the rhythm of IOP. Male New Zealand White rabbits were used and entrained to a lighting schedule of alternating 12-hour periods of light and dark for at least two weeks prior to unilateral preganglionic section of the cervical sympathetic trunk. IOP was measured with an applanation pneumatonometer. IOP in rabbits has a circadian rhythm and is not a direct response to environmental lighting conditions. Lee et al. (1995) demonstrated that exposing rabbits to light during the dark phase of the circadian cycle produced a rapid, reversible decrease of IOP.

A circadian IOP pattern is also prevalent in cats. Del Sole et al. (2007) undertook a study to investigate the 24-hour IOP pattern in cats maintained under a 12-hour light and 12-hour dark cycle. Del Sole et al. (2007) determined that higher IOP values were obtained during the night phase than during the light phase, supporting the theory that cats contain an endogenous circadian rhythm. The study also demonstrated that age and sex of the species did not have an affect on IOP. Yet, previous studies have indicated a decrease in IOP with cat age (DelSole et al.,
Even though the study aimed at identifying 24-hour IOP variation in cats with ocular diseases, only a small number were present. Two cats were identified as having uveitis and one cat with glaucoma. The cases with uveitis and glaucoma showed that IOP values were also higher during the dark phases.

Although there are several promising animal models of glaucoma that have provided valuable information about the disease (Guo et al., 2011; Morrison et al., 2011; Howell et al., 2008), none have so far been used to explore diurnal variation in IOP. In summary, studies have shown that rats, rabbits and cats, entrained to a 24-hour lighting cycle demonstrate a circadian rhythm with which IOP coordinates to the beginning of light and dark phases.

1.3.4 IOP Measuring Techniques

1.3.4.1 Applanation Tonometry

Tonometry is a method of indirectly measuring IOP. Applanation tonometry measures IOP by determining the pressure required to flatten or applanate the cornea or sclera. Applanation tonometry is based on the Imbert-Fick Law where a pressure within a sphere is roughly equal to the external force needed to flatten a proportion of the sphere divided by the area of the sphere that is flattened:

\[ P = \frac{f}{A} \]

\( P = \text{Sphere} \)
\( f = \text{Force} \)
\( A = \text{Area} \)

Where \( P \) is sphere, \( f \) is force and \( A \) is the area. The law assumes that the sphere lacks rigidity as well as thickness. The most basic form of applanation tonometry by contact is the Goldmann Applanation Tonometry that measures the amount of applanation of the ocular surface in response to a given force.
1.3.4.1.1 Contact Tonometry (Goldmann Applanation Tonometry)

The gold standard for clinical measurement of IOP is the Goldmann Applanation Tonometer (GAT). The GAT consists of a cylinder that rests on top of a rod whose base is attached to a spring with a precise force. The applanating surface of the GAT has a fixed area of $7.35 \text{ mm}^2$ with a diameter of 3.06 mm. The diameter was chosen at 3.06 mm so that the forces of tear surface tension, the applanating area and the corneal elasticity, cancel each other: the force applied by the spring in grams when multiplied by 10 is equivalent to the IOP in mmHg. A cobalt blue light activates fluorescein dye that alters the tear layer to a greenish color. At the point of measurement, a meniscus at the edge of the applanated corneal surface is formed when the tear layer is depressed out from between the applanating surface of the tonometer tip and the cornea. This circular meniscus is then divided into a superior and inferior arc and its apposition determines whether the IOP is high, low or normal (Figure 1-5).

Although the GAT is the standard method for measuring IOP there are several disadvantages to using this type of tonometer. First, the GAT is dependent on corneal thickness, corneal curvature, and axial length, overestimating IOP for individuals with thicker corneas and underestimating for individuals with thinner corneas (Norman et al., 2010; Asejczyk-Widlicka and Pierscionek, 2008; Patwardhan et al., 2008; Suman et al., 2013). This can cause misclassification of POAG patients with thin corneas as having NTG and can also misclassify normal patients with thick corneas as having ocular hypertension (ElMallah and Asrani, 2008). The slit lamp mounted GAT is non-portable and used only to measure IOP while the patient is awake in the upright position.
However, the Perkins tonometer, the portable counterpart to the GAT, is able to measure IOP in the supine position. Correction tables for CCT when using GAT have been proposed, but are generally not advised for clinical practice. Repeated tonometry may also induce decline in estimated IOP because of the constant applanation of the cornea. Nevertheless, the GAT measures IOP within an error of 1 mmHg (Chihara, 2008). Other disadvantages associated with the GAT include controlling the level of fluorescein in the tear film, high astigmatism, irregular corneas, and pressure from a finger on the eye or eyelid while taking the measurement (Stamper, 2011).

1.3.4.1.2 Non-Contact Tonometry (Airpuff)

Non-contact tonometry was initially developed for scenarios where anesthetic was not available for use. Like the GAT, the airpuff (AP) uses a column of air to applanate the cornea. As the cornea is deformed as the air column is emitted, the curved corneal surface reflects light to a detector. Thus, corneal applanation is measured by evaluating the point that the angle of incidence equals the angle of reflection (Farhood, 2013). The angle of reflection will be the strongest at an angle when the cornea is completely flat and acts like a plane mirror (Farhood, 2013). Although the AP has advantages, including that it does not require topical anesthetic, does not cause corneal abrasion and requires no sterilization because the applanation surface is a column of air, there are several disadvantages to using this type of tonometer. First, the readings are instantaneous and simply provide a snapshot reading of IOP (Stamper, 2011), it tends to underestimate elevated IOP (Moseley et al., 1989) and may be more influenced by CCT than the GAT but not the Ocular Response Analyzer, which is the most current form of airpuff tonometry (Stamper, 2011).

1.3.4.1.2.1 Ocular Response Analyzer

The Ocular Response Analyzer (ORA) is a type of airpuff tonometry and measures IOP free from the influence of corneal biomechanical factors. The ORA characterizes corneal parameters by applanating the cornea at two different time points. The difference in these two points provides measurements of the viscoelastic properties of the cornea. The ORA is able to measure corneal hysteresis, a GAT correlated IOP and a corneal compensated IOP (ElMallah and Asrani, 2008). A study conducted by Medeiros and Weinreb (2006) demonstrated that the ORA was less influenced by corneal parameters than the GAT in patients with no indication of glaucoma. Yet,
a study conducted by Martinez-de-la-Casa et al. (2006) found that the ORA overestimated IOP when compared to GAT in patients with glaucoma.

1.3.4.2 Indentation Tonometry

Indentation tonometry is based on the principle that a given force will indent into a soft object further than into a hard one. The simplest example of indentation is by taking a finger and pressing it against the eyelid; if the pressure is low the finger will indent farther than that of a hard eye that would have a higher pressure. Indentation tonometry was initially used on the sclera until the introduction of topical anesthesia that allowed the cornea to be used as the principle site of IOP measurement. This was most probably because the cornea was more easily accessible than the sclera and measurements could be acquired without lifting the lid. There are several indentation tonometers that are currently used in the clinical setting such as the Tonopen, Rebound tonometer, and Dynamic Contour Tonometry. The Schiötz tonometer, no longer in common use, was the standard instrument for tonometry for many years.

1.3.4.2.1 Schiötz Tonometer

The Schiötz tonometer consists of a plunger with weights attached to a footplate that is molded to the curvature of the cornea. Indentation of the anesthetized cornea by the plunger causes a needle to move across a linear scale that represents logarithmic values (Moses, 1971). A conversion table is then used to convert the scale reading into IOP mmHg. The Schiötz tonometer has to be calibrated at each use by placing it on a steel block in order for the scale to read “0”. The advantages to this type of tonometry include its portability and the fact that it is easy to sterilize. However, it also has many disadvantages that make it impractical for use. This type of tonometer can only be used in the supine position. Most importantly, the patient has to be extremely still during measurements, as eye movements may render a corneal abrasion. When the tonometer is placed on the eye, its weight is enough to displace aqueous humor, initially raising the IOP above baseline (Stamper, 2011). If repeated measurements are taken, aqueous humor is again displaced causing a decrease in IOP. Specific calculations must be made in order to account for this error. Elasticity of the eye is also underestimated when measured with the Schiötz tonometer. Individuals with myopic eyes have low ocular rigidity causing IOP to be underestimated in comparison to individuals with hyperopic eyes that have higher ocular rigidity.
It is rare for the Schiotz tonometer to be used in contemporary ophthalmic practice.

### 1.3.4.3 Tonopen

The tonopen combines indentation and applanation principles, measuring IOP over a brief interval. The tip of the tonopen is attached to a pressure transducer, once applied to the cornea, the pressure gradually increases and the response is transferred to a plate that outputs the IOP in mmHg. It is small, portable and able to record in the supine or sitting position. The tip of the instrument is covered with a disposable silicon cover that requires it to be replaced each time after use to prevent transfer of infection. However, this adds to the cost of pressure measurements, along with the need to replace batteries. Like other tonometers, it measures IOP over a brief interval, and so several readings need to be averaged to reduce the effects of the cardiac and respiratory cycles. Several studies have been performed to determine the comparison of the tonopen and the GAT. Kao et al. (1987) demonstrated that the Tonopen tended to underestimate GAT’s IOPs if they were above 21 mmHg and overestimate IOP if they were less or equal to 9 mmHg. Other studies have shown good correlation between the two types of tonometers (Hines et al., 1988; Alfaro & Tran, 1991), however correlation does not require concordance. A study by Horowitz et al. (2004) suggested a good comparison between the tonopen and the GAT if the Tonopen measurements were taken more than 2 times per eye and then averaged (Horowitz et al., 2004).

### 1.3.4.4 Rebound Tonometer

Rebound tonometers use an induction current to measure IOP. This is accomplished by bouncing a small plastic tipped metal probe against the cornea. ICare consists of a 1.8 mm diameter plastic ball on a stainless steel wire held in place by an electromagnetic field in a hand held device (Stamper, 2011). Initially used for small animal research as topical anesthetics are not required, because the tip only makes contact with the cornea for microseconds (Kontiola & Puska, 2004). In addition, ICare has the advantage of being portable, easy to use by non-professionals, and well tolerated by young children. It has also shown good correlation with the GAT and tonopen, although readings were about 1.5 mmHg higher than the GAT (van der Jagt & Jansonius, 2005). Bruisini et al. (2006) compared the ICare tonometer with the GAT in 178 patients with glaucoma. Measurements were affected by CCT but correlated to those measured with the GAT.
(Brusini et al., 2006). Another study reported similar results when comparing the ICare tonometer to the GAT, also establishing that patients had no preference as to which tonometer was more comfortable (Vandewalle et al., 2009). Recently, Rosentreter et al. (2011) compared a new rebound tonometer for in-home IOP monitoring. Measurements on 126 eyes were taken by three different ophthalmologists with GAT, compared to the in-home tonometer, the ICare ONE rebound tonometer (RTONE), measured by the study participants. Seventy-five percent of the patients were able to accurately measure their own IOP with the RTONE and were able to show a correlation with those measurements obtained with the GAT (Rosentreter et al., 2011).

1.3.4.5 Dynamic Contour Tonometry

Dynamic Contour tonometry (DCT) is a newer form of tonometry that was first described in 2005 by Kanngiesser et al. (Kanngiesser et al., 2005). It uses the principle of contour matching rather than applanation to measure IOP. DCT is used to diminish the effect of corneal parameters such as curvature and thickness, which can reduce the accuracy of GAT (Doyle and Lahkar, 2005; Kotecha et al., 2005). The tip contains a cup-like plastic device that matches the curvature of the cornea with a piezoelectric pressure sensor in its centre. Rather than applanating the cornea, DCT is influenced by corneal curvature and avoids deforming the cornea during measurements. This allows it to be more independent of corneal parameters than the GAT (Doyle & Lachkar, 2005; Schneider & Grehn, 2006). The DCT is able to yield pressure curves that can determine the ocular pulse amplitude. The ocular pulse amplitude is a value that represents the difference between the average systolic IOP and the average diastolic IOP. Current theory states that the ocular pulse amplitude represents a true indirect measure of intraocular pulsatile blood flow because it is generated by the filling of the choroidal vasculature during the systole (Knecht et al., 2012). A study conducted by Moghimi et al. (2013) compared the IOP and ocular pulse amplitudes in patients with POAG to evaluate ocular and systemic factors linked to the ocular pulse amplitude. The study confirmed that the DCT did not correlate with central corneal thickness for IOP measurements. Disadvantages of the DCT include the cost of the device, the cost of the disposable tips, and the need for more patient cooperation than other tonometers (Stamper, 2011).
1.3.5 Continuous Measurement of IOP

The different methods for measuring IOP described above only provide partial knowledge of a patient’s true IOP because of their inability to continuously measure it. IOP has been shown to elevate during nocturnal hours for humans (Aref, 2013; Konstas et al., 2012), likely due to an increase in episcleral venous pressure and vascular recirculation in the supine position (Weinreb & Liu, 2006). To date, there is no commercially available device invasive or non-invasive that measures IOP continuously while sleeping and/or in the supine position. Clinicians have occasionally reported on 24-hour phasing, but a study conducted by Moodie et al. (2010) showed that 24-hour phasing offered little benefit over daytime phasing in recognizing IOP peaks. They also acknowledged that it is difficult not to stimulate the patient during sleep while taking a recording. In addition, inter-individual and intra-individual variation in corneal thickness and ocular rigidity may result in significant deviations in applanation-based methods of measuring IOP (Bhan et al., 2002; Tonnu et al., 2005; Harada et al., 2008).

Table 1-1. List Of Invasive And Non-invasive Continuous IOP Monitoring Devices.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Monitoring Device</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collins et al.</td>
<td>1967</td>
<td>Miniature passive pressure transensor for implanting in the eye</td>
</tr>
<tr>
<td>Greene &amp; Gilman</td>
<td>1974</td>
<td>Instrumented contact lens</td>
</tr>
<tr>
<td>Cooper et al.</td>
<td>1979</td>
<td>Scleral ring guard</td>
</tr>
<tr>
<td>McLaren et al.</td>
<td>1996</td>
<td>Implanted pressure transducer in the anterior chamber of pigmented rabbits</td>
</tr>
<tr>
<td>Schnell et al.</td>
<td>1996</td>
<td>Implanted transmitter in the superior conjunctival sac and inserted in to the midvitreous in albino rabbits</td>
</tr>
<tr>
<td>Leonardi et al.</td>
<td>2004/2009</td>
<td>Triggerfish SENSIMED contact lens IOP monitoring device</td>
</tr>
</tbody>
</table>
Various sensor devices and both invasive and non-invasive technologies for measuring continuous IOP have been published (Collins et al., 1967; Greene & Gilman, 1974; Cooper et al., 1979; McLaren et al., 1996; Schnell et al., 1996; Leonardi et al., 2004; Leonardi et al., 2009). Over the years an attempt has been made to develop a device that can accurately measure 24-hour IOP in order to aid in the management of glaucoma. Continuous IOP monitoring would be favorable in order to obtain a better understanding of IOP in glaucomatous eyes over the 24-hour period. Temporary IOP monitoring results in difficulty with noise and artifactual IOP readings since movement of the sensor with respect to the eye would likely be a significant source of noise. Also, these systems are not successful due to the fact that the signal strength lessens with working distance. Non-invasive as well as invasive devices have been introduced. The most challenging aspects of these devices are not only location of where they will be placed in the eye, but most importantly is to ensure their long-term biocompatibility.

In 1974, Greene and Gilman introduced the first non-invasive method for monitoring IOP (Greene & Gilman, 1974). Their system consisted of a soft contact lens with a strain gauge sensor that measured changes in IOP by sensing the deformation of the meridional angle of the corneoscleral junction in a rabbit model (Greene and Gilman, 1974). Greene and Gilman (1974) hypothesized that the deformation in the angle where the cornea joins the sclera may vary more in response to IOP changes than other ocular structures. The medical device was fitted to the corneoscleral junction to obtain maximum output with contained strain gauge transducers imbedded in it. The lens was made from Dow Corning Medical Grade Polydimethylsiloxane. It was linear over the range of 20 to 57 mmHg with a slope approximately 0.08 ohms per mmHg. The preliminary data of the study supported their assertion that a strain gauge embedded in a soft contact lens could measure changes in IOP via deformation of the meridonal angle of juncture between the cornea and sclera.

In 1979, Cooper et al. used a continual scleral guard ring applanation transensor to measure IOP in rabbits and dogs (Figure 1-6) (Cooper et al., 1979). By means of passive radiotelemetry, a miniature scleral applanating device was embedded in a haptic contact lens. Experimentation was performed in vivo in rabbits and dogs, producing consistent linear outputs in both species. Reproducibility of the device diminished with time and several problems affected the stability of the device. These included temperature, ambient temperature, coupling of the device to the eye, physical properties of the sclera, mechanical instability within the device, permeability to saline
and the geometric relationship between the device and the aerial system (Cooper et al., 1974). Although small changes in body temperature and ambient temperature did not affect the recordings of the scleral guard ring, linear output diminished with time. Multiple regression analysis of calibration studies performed in the rabbit model identified that the scleral ring guard was not affected by minute changes in body temperature. However, the same analysis was not true when applied to the canine model because of lower ocular rigidity. The authors argued that since the human scleral rigidity lies between that of the rabbit and the canine, a considerable analysis of the effects of continuous scleral applanation would have to be tested in human eyes.

Figure 1-6. Illustration of the Scleral Ring Guard. The scleral ring guard was a miniature scleral applanating device, mounted in a haptic contact lens that was devised by Cooper in 1974. The principle of the applanating transensor was based on resonant frequencies that altered with scleral deformation. Several factors affected the accuracy of the applanating transensor that included (1) temperature; (2) atmospheric pressure; (3) coupling to the eye; (4) physical properties of the sclera; and (5) mechanical instability. (Cooper et al., 1974; ©ARVO).
In 1967, Collins et al. described an implantable device for continuous measurement of IOP using an implantable pressure sensitive radio transensor using a pair of parallel spiral coils, where a change in IOP induced a shift in the resonant frequency (Collins et al., 1967). Several groups have reported an IOP monitoring system that consisted of a pressure sensor and radio frequency oscillator on a single chip resulting in accurate recordings (Stangel et al., 2001; Walter et al., 2000). McLaren et al. (1996) were the first group to continuously or permanently measure IOP in conscious unrestrained rabbits using a commercial, implanted device. This allowed for continuous IOP monitoring throughout the 24 hours for several months. Yet, the results were limited by the battery life of the transducer. A similar device was also created by Schnell and colleagues in 1996 (Schnell et al., 1996). A transmitter was placed under the skin and a catheter was directed subcutaneously to the superior conjunctival sac and inserted into the midvitreous. Correlation with IOP in the anterior chamber was performed by pneumatonography measurement and by manometric pressure perfusion (Schnell et al., 1996). They were able to demonstrate the practicability of measuring IOP over an extended period of time. Thus, there have been several attempts to find a practical and portable solution for IOP monitoring without success (Walter et al., 2000; Collins, 1967; Greene & Gilman, 1974; Cooper et al., 1979).

More recently, Leonardi et al. (2004) employed a soft contact lens made of silicone with an embedded micro gauge to measure IOP via corneal curvature. They went on to develop a wireless silicone contact less sensor for continuous monitoring of IOP (Leonardi et al. 2009). The device was adapted and tested on enucleated pig eyes and demonstrated minimal invasiveness and functionality in monitoring IOP (Leonardi et al., 2009). The device was based on the assumption that central corneal radius of curvature changes by approximately 3µm for every 1mHg change in IOP in enucleated pig eyes (Leonardi et al., 2004; Leonardi et al., 2009) (Figure 1-7). The contact lens sensor showed high linearity ($r^2=0.993$), a sensitivity of $109\mu V/mmHg$ and a reproducibility of ±0.2mmHg. The device demonstrated reproducibility and the ability to measure a signal equivalent to the human ocular pulsation (Piso et al., 2012). The primary design of Leonardi’s contact lens used a wire lead from strain gauges that were connected to a recording device that encircled the upper and lower eyelid. Later refinements of the design led to a microfabricated strain gauge in a Wheatstone bridge configuration with two sensing resistive gauges and two compensative resistive gauges. The resistive gauges would double sensitivity and the compensative resistive gauges would compensate for thermal drift.
The sensing resistive gauges in the device were designed to have a circular arc with a diameter of 11.5mm, equivalent to the average corneoscleral junction (Piso et al., 2012). Changes in IOP would cause the maximum corneal deformation at the corneoscleral junction and therefore the meridional angle of that site was chosen as placement for the contact lens sensor (Leonardi et al., 2009). The strain gauge sensor was based on a polyimide-platinum-polyimide (PI-Pt-PI) film with gold loop antennas (Leonardi, 2007). Platinum and polyimide materials were employed because of their flexibility, low water uptake, biocompatibility, thermal stability and insulating properties. Gold was also chosen for the antennas because of its low resistivity and flexibility in being conformed to the shape of the contact lens (Leonardi, 2007).

The contact lens material of Leonardi’s contact lens sensor was composed of pure silicone, a hydrophobic material. A pure silicone contact lens has the drawback of adhering to the corneal surface, potentially causing corneal swelling. In order to prevent silicone contact lenses from adhering to the corneal surface, they have to be surface treated to make them hydrophilic and improve comfort. The contact lens device that was developed by Leonardi et al. (2009) was not surface treated because of the high cost required to do so. Also, since the device would be disposable and only used for a 24-hour period, indications of surface treating were inapplicable.

Mansouri and Shaarawy (2011) presented results of a 24-hour human trial using a wireless version of the device, Triggerfish® (SENSIMED, Switzerland), in 13 patients with open-angle glaucoma. The authors reported the ability to monitor IOP fluctuations in patients over 24-hours with no adverse reactions other than keratitis in four patients (31 percent) (Mansouri & Shaarawy, 2011). De Smedt et al. (2012) recently released their study evaluating patient comfort
and accessibility of Leonardi’s corneal silicon based contact lens IOP sensor (SENSIMED Triggerfish®). The study consisted of 10 healthy control subjects who would were assessed after wearing the sensor for 5 and 30 minutes, 4, 12 and 24 hours. The sensor used in this study was approximately 14.4 mm and 8.7 mm and its thickness was 100 µm at the border and 600 µm at the centre (De Smedt et al., 2012). Comfort scores were obtained subjectively and based on a scale of zero to 10, zero being intolerable and 10 perfect. The normal subjects were asked to record their activities in a logbook to be able to connect their actions to the changes recorded in the sensor. The normal subjects also underwent a full eye examination which consisted of an evaluation of best-corrected visual acuity, slit lamp, GAT, pachymetry, automatic keratometry, measurement of pupil and horizontal corneal diameters, gonioscopy and fundoscopy. One participant experienced a significant reduction in best-corrected visual acuity during sensor wear; however, all subjects reported a high comfort wear over the 24-hour period (De Smedt et al., 2012). Pajic et al. (2011) evaluated continuous IOP fluctuation recordings in NTG patients with the SENSIMED Triggerfish® device. They performed 24-hour IOP fluctuation monitoring in five NTG patients in the presence and absence of anti-glaucoma pharmacotherapy in order to demonstrate the clinical importance of the contact lens monitoring device. Their study concluded that the contact lens device was able to monitor IOP rhythms and differences associated with anti-glaucoma medicine (Pajic et al., 2011). Recently, Mansouri et al. (2012) evaluated the safety, tolerability, and reproducibility of the Triggerfish® SENSIMED in 21 individuals who were glaucoma suspects and in 19 individuals with established glaucoma. The mean age of their research group was 55 years of age and 60 percent were male. The main adverse reactions included blurred vision in 82 percent, conjunctival hyperemia in 80 percent, and keratitis in 15 percent. The study concluded that the Triggerfish® SENSIMED contact lens showed fair to good repeated patterns of IOP monitoring and that the repeated use of the contact lens sensor demonstrated good safety and reproducibility (Mansouri et al., 2012). Yet, a recent study conducted by Holló and colleagues (Holló et al., 2013) demonstrated the non-utility of the Triggerfish® SENSIMED contact lens to detect changes in IOP during and after therapy with a synthetic prostaglandin analog, travoprost. The study evaluated 24-hour continuous monitoring of IOP for assessment of prostaglandin-induced pressure reduction in patients with ocular hypertension (n=4) and POAG (n=5). Patients were investigated after washout from IOP lowering treatment and then under travoprost monotherapy. The 24-hour IOP profile measured by the Triggerfish® SENSIMED contact lens showed no difference before or after therapy, whereas
the Goldmann Applanation Tonometer was able to measure significant change in IOP. The Triggerfish® SENSIMED contact lens was also unable to show measurement differences in IOP between various postural positions, such as sitting, supine or walking. The results of the study suggested that the Triggerfish® SENSIMED contact lens was unable to identify transient IOP elevation periods (Holló et al., 2013). Moreover, the study found that all Triggerfish® SENSIMED contact lens measurements increased over time worn by the patients, with a possible explanation being thermal drift or strain gauge hysteresis.

Large clinical trials using the Triggerfish® SENSIMED contact lens device remain to be performed, and the ability to correlate corneal curvature and IOP remains to be established. Moreover, because the strain gauge is embedded in a silicon contact lens, it can be subject to significant motion artifacts unless shaped to fit each individual’s cornea, a concept that was first introduced by Greene and Gilman (Green and Gilman, 1974) when they proposed their soft contact lens IOP monitoring device some 30 years ago. Inter-individual and intra-individual variation in central corneal thickness and rigidity, particularly in patients with corneal irregularities could result in measurement error or deviations when relying on measuring IOP via corneal curvature (Browning et al., 2004; Doughty et al., 2004). Kida et al. (2006) documented on the circadian variation of corneal thickness, concluding that CCT was thicker during the nocturnal period than during the diurnal period (Figure 1-8). The nocturnal period resulted in swelling of the cornea due to requiring a higher hydration state. It is also unclear whether patient comfort has been investigated especially if the patient has to wear a wired contact lens. Another issue that has not been addressed by Leonardi and colleagues regarding the SENSIMED Triggerfish® is the thermal drift of the contact lens and how it affects measurement accuracy. No mention has been given regarding the strain gauge material and thermal compensation. Thermal compensation is a crucial aspect of medical device stability and may render a device unsafe for ophthalmic use. The thermal compensation of a strain gauge refers to the change in sensitivity of the device to strain with alteration in temperature. Alterations in temperature may result in a serious of effects that cause the result in error. For instance, an inability to correct for thermal compensation may result in thermal expansion of the strain gauge resulting in a resistance of the strain gauge as well as the connecting wires to change and provide false measurements.
Figure 1-8. 24-hour Variations of Central Corneal Thickness, IOP and Corneal Hysteresis in Healthy Young Patients. Measurements of central corneal thickness were performed with an ultrasonic pachymeter and measurements of IOP and corneal hysteresis with an Ocular Response Analyzer (Kida et al., 2006; ©ARVO).

In summary, to date no devices have been developed that are non-invasive, provide 24-hour monitoring of IOP in humans, during sleep under closed eye conditions that are commercially available in North America. Several devices have been developed that have been listed in Table 1-1, yet only the contact lens device developed by Leonardi et al. (2009) (Triggerfish®) has demonstrated the ability to produce recordings that are continuous throughout the day and night cycle. The Triggerfish® is commercially available in Europe and is currently awaiting Food and Drug Administration (FDA) approval in the United States. However, no large clinical studies have been performed to fully establish its safe use for overnight corneal contact lens wear in a glaucoma population.

The dynamic nature of IOP behavior particularly at night when IOP tends to increase renders the crucial need for an IOP continuous monitoring device. Such a development will be important in understanding and managing glaucoma.
1.4 Strain Gauge

1.4.1 Strain Gauge and Electrical Resistance

Strain gauges are devices that measure the strain (normalized deformation) as in the change in length in compared to the original length, or the stress (normalized load) as in the force over a certain area, in a given material. The extension of the strain gauge for a given load varies with the geometry of the material tested as well as its composition. As a material deforms in response to a normalized deformation or normalized load the strain gauge is deformed causing a change in its resistance. This change in resistance is related to the strain and is referred to as the gauge factor. The common principle of a strain gauge is that the electrical resistance of a length of wire is directly proportional to the change in any applied strain or stress applied to it.

![Figure 1-9. Strain Gauge Dynamics](image)

Dynamics of a bonded strain gauge expressing the relationship between the compression and the resistance.

The most effective way of measuring electrical resistance of a strain gauge is by a Wheatstone bridge. A Wheatstone bridge is an electrical circuit that is employed to measure an unknown electrical resistance by balancing the bridge circuit. The bridge circuit is generally arranged with four arms that are interconnected (Ra, Rb, Rc and Rx) (Figure 1-10). Among these arms are two known fixed resistances known as ratio arms. If the ratio of the two arms or resistances, Rb/Ra, is equal to the ratio of the two Rx/Rc, then the voltage across the bridge Vb will be zero. The value of the output voltage depends on the ratio of the resistors. The Wheatstone bridge is well
suited for the measurement of small changes in resistance in a strain gauge that then electronically convert this measure into a pressure reading.

![Wheatstone Bridge Configuration](image)

**Figure 1-10. Wheatstone Bridge Configuration.** Illustration of a Wheatstone bridge configuration that connects two parallel arms, containing four resistors, three of which are of known value. One arm contains one known resistance and an unknown ($R_x$). The other parallel arms contain resistors of known resistances. In order to determine the resistance of the unknown resistor, the resistances of the other three are adjusted and balanced until the current passing through decreases to zero.

1.4.2 Thermal Output

Ideally, a strain gauge bonded to a material would respond solely to the applied strain and be unaffected by other variables present in the environment. Unfortunately, the electrical resistance of the strain gauge varies not only with the strain or stress of the material, but also with the temperature. In addition, the gauge factor that corresponds to the relationship between the strain and resistance change varies with temperature. This variation with temperature can cause significant errors if not properly corrected. Once a strain gauge is installed to a transducer and is balanced with the addition of a Wheatstone bridge circuit, a subsequent change in temperature may be produced as a result of resistance change in the gauge. This temperature induced resistance change is not related to the stress or strain of the material that the strain gauge is bonded to, but merely a temperature change that corresponds to the thermal output of the strain gauge. Thermal output is potentially the main source of error in strain gauge measurements.

When measuring at room temperatures, the error from thermal output, if not properly controlled, may be larger than the magnitude of the load to be measured. Thus, it is essential to provide some source of thermal compensation to correct the strain measurements. Many factors affect the thermal output of the strain gauge. These include but may not be limited to the test material and its shape, the grid alloy, bonding and encapsulating materials and installation procedures.
The main cause of thermal output stems from the basis that the electrical resistivity of the grid conductor is temperature dependent. As a result, the gauge resistance varies with temperature. A secondary cause of thermal output may be due to the differential thermal expansion between the grid conductor and the material to which the strain gauge is adhered. When the temperature changes it causes the material to expand or contract. Since the strain gauge is firmly bonded to the material, the gauge grid is forced to undergo the same structural changes causing the thermal expansion coefficient of the gauge grid to deviate from that of the material. Therefore, the gauge grid is mechanically strained in trying to conform to the expansion and contraction of the material. The gauge grid is strain sensitive by design and this results in the strain gauge displaying a resistance change proportional to the differential expansion of the material.

Thermal output can be compensated for and eliminated by connecting to the Wheatstone bridge circuit an adjacent arm of identical compensation or a “dummy” gauge. A “dummy” gauge compensates for the thermal strain by removing erroneous signal from the output of the bridge only if the thermal output is identical for the active and compensating gauge (Scalea, 1998). If the two gauges both active and compensating have the same thermal output, then the resistance changes in adjacent arms of the Wheatstone bridge circuit will not unbalance the circuit and the thermal output of the those two gauges should cancel each other out. Because the temperature changes are identical in the two gauges, the ratio of their resistance does not change as well as the voltage, and the effects of the temperature change are diminished. This would result in the strain gauge only recording the induced load (stress or strain) on the material. By compensating for thermal output via a compensating gauge a quarter-bridge is formed. Alternatively, the sensitivity of the bridge to strain can be doubled by making both gauges active in alternating directions forming a half-bridge. The half-bridge circuit will yield an output voltage that is linear and approximately doubles the output of the quarter-bridge circuit.
1.4.3 Pre-Wired Strain Gauge

The strain gauge sensor used as proof-of-concept (Chapter 2 and Chapter 3) is a pre-wired gauge commercially available from OMEGA Engineering (KFG-5-120-C1-11L1M2R) (Figure 3-1). The gauges have a linear pattern with medium length grids, three-meter leads and 120 Ohms (Ω) of resistance. Their measuring grid is constructed from Constantan foil with a polyimide carrier material and measure 9.4 x 2.8 mm in length. Gauge Factor Tolerance percent ±1, with 1.5 mm grid length percent ±1.5, temperature coefficient of gauge factor 1/K [1/°F] (115 ± 10) x 10-6 [(64 ± 5.5) x 10-6, nominal value of gauge factor temperature coefficient.

There is a linear relationship between strain and resistance variation of the strain gauge that is expressed as:

\[
K = \frac{\Delta R}{R} = \frac{\Delta R}{R} \frac{\Delta L}{L} \epsilon
\]

In general, the strain of the tissue (i.e. sclera, conjunctiva) causes a change in length in the wire of the strain gauge that ultimately causes a change in electrical resistance. Ideally, it would be beneficial if the resistance of the strain would alter only in response to applied strain. However, as previously stated, the strain gauge as well as the tissue will also respond to variations in temperature. The strain gauge has been previously tested by the manufacturer for its thermal output and is stated as ±0.85 [(µm/m)/°C]. Thus, when using a two-wire strain gauge, temperature changes will cause a zero shift beyond what is computed. For two wire gauges the zero drift due to lead wire may be computed by the following equation:

\[
\epsilon l = \gamma \alpha \Delta T \frac{K_s R}{K}
\]

\(\epsilon l\) = Zero strain drift due to lead wire changing temperature  
\(\gamma\) = Resistance of lead wire alone exposed to a temperature change (ohms) 
(wire supplied is 0.22 ohms/meter, therefore 2 leads, 1 meter long, \(\gamma = (0.22)(1)(2) = 0.44\) ohms)  
\(\alpha\) = Resistance temperature coefficient of lead wires
(copper wire = 0.0038/°C)

\[ R = \text{Total resistance value of gauge including lead wires (ohms)} \]

\[ \Delta T = \text{Temperature change (°C)} \]

\[ K_s = \text{Gauge factor} = \frac{(R)(K)}{(R - \gamma)} \]

\[ K = \text{Total gauge factor including lead wires} \]

Therefore, for a 2 wire lead gauge undergoing a 5 °C change with lead wires 1 meter long:

\[ \varepsilon \ell = (0.44)(0.0038/°C)(5°C) = 33 \times 10^{-6} \text{ m/m or } 33 \mu \varepsilon \]

\[ (2.107)(120) \]

(strain is often expressed as microstrain (µε) which is e x 10^-6)

Where:

\[ \gamma = 0.44 \text{ Ohms} = (0.22)(1)(2) \]

\[ \alpha = 0.0038/°C \]

\[ \Delta T = 5°C \]

\[ R = 120 \text{ Ohms} \]

\[ K_s = \frac{(R)(K)}{(R - \gamma)} = \frac{(120)(2.10)}{(120 - 0.44)} = 2.107 \]

\[ K = 2.10 \]

Several factors had to be in place to properly ensure that the pre-wired strain gauge was working and installed correctly. These included the following: (1) inspecting the test material and the location to which the strain gauge would be adhered. The material tested was scleral and conjunctival tissue in Chapter 2 and the scleral, bulbar conjunctiva in a live animal model in Chapter 3; (2) Preparing the strain gauge by exercising prior to recording; (3) Preparing the material for surface cleaning in order for the strain gauge to properly adhere. This required that the ocular tissues or surface be gently wiped down dry. Cyanoacrylate was used in both Chapter 2 and Chapter 3 to adhere the strain gauge to the ocular tissue or surface; (4) Properly positioning the strain gauge on the material and having remain bonded for the length of experimentation; (5) Inspecting the installation and checking for resistance and wiring before recording.
1.5 Rationale for a “Scleral” Sensor

The conceptualization for placing the strain gauge on the sclera rather than the cornea to measure IOP over extended periods of time is based on the material properties of the sclera and its relationship with IOP. The sclera is a fibrous, collagenous structure that composes the posterior 80 percent of the eye (Newell, 1986). Anteriorly, the sclera is covered by the vascular episclera, Tenon capsule and the conjunctiva. Historically, the sclera was the ocular site for IOP measurements by indentation tonometry. However, the introduction of topical ophthalmic anesthesia opened the way for measuring IOP from the cornea, which proved to be much easier as indentation or applanation could be applied directly there (Stamper, 2011).

Lowering IOP has proven to be the only therapeutic method of preventing or halting the progression of glaucomatous vision loss (Burgoyne et al., 2005; Sigal et al., 2005; Sigal et al., 2009). However, the role of IOP in glaucoma is still unclear. Several theories of pathogenesis have been mentioned, all implicating an association between changes in IOP and loss of retinal ganglion cell axons resulting from the biomechanical rearrangement within the optic nerve head and the lamina cribosa (Sigal et al., 2011). Experimental studies have shown that as IOP varies, the sclera deforms substantially playing an important role in the response of the lamina cribosa to IOP (Sigal et al., 2011; Sigal et al., 2005; Sigal et al., 2004). Sigal et al., (2011) have reported that the peripapillary sclera and the optic nerve head, in response to IOP changes, act as a mechanical system that affects the lamina cribosa. Such an interaction then may contribute to the disruption of the retinal ganglion cells axons consequentially resulting in glaucomatous vision loss. Sigal et al. (2011) used parameterized eye-specific baseline models of the lamina cribosa and sclera of normal monkeys to analyze acute changes in IOP in the anterior-posterior lamina cribosa deformation and the scleral canal expansion. The study illustrated that the effects of IOP on the optic nerve head are a result of several factors that include the geometry and material properties of the lamina cribosa, in terms of position, and sclera, in terms of thickness (Sigal et. al., 2011). Thus, a theoretical argument can be implied that individuals with a thicker sclera are less prone to glaucomatous optic neuropathy, implicating that the sclera could be a potential biomarker for the assessment of developing glaucoma (Sigal et al., 2011; Quigley and Cone, 2013).
Individual variations in the sclera and its response to IOP elevations are likely to be important risk factors for glaucoma. Crawford-Downs et al. (2007) demonstrated that the biomechanical response of the posterior sclera is an important determinant of strain at the ONH because of the tight coupling between the sclera and lamina cribosa. Variations in the mechanical properties of the scleral could provide insight as to why fifty percent of the individuals with open angle glaucoma are susceptible to injury in the normal IOP range (Quigley and Broman, 2006). Quigley and Cone (2013) cited that the susceptibility to the effects of IOP in glaucoma may be diminished by altering the properties or responses of the sclera. The study proposed that treatments could aim at changing the mechanical response to IOP by altering the baseline state of the sclera (Quigley and Cone, 2013). No specific therapies were documented but the potential treatments for glaucoma that target the sclera are supported by illustrating that eyes with mechanical responses that are characterized as “stiffer” are less susceptible to glaucomatous optic neuropathy (Quigley and Cone, 2013; Sigal et al., 2011). Other therapeutic targets to alter the susceptibility to glaucomatous optic neuropathy may exist in pathways that are related to scleral remodeling. Proteomic findings by Quigley and Cone (2013) illustrated that changes in the sclera occur quickly after IOP elevations. More specifically, substantial changes in the activity of scleral fibroblasts were documented after increases in IOP indicating a transition to the myofibroblast phenotype among scleral cells.

IOP generates a stress on the eye wall affecting both the cornea and sclera causing a pressure differential across the optic nerve head that contributes to the development of optic nerve cupping (Quigley and Cone, 2013). Yet, in comparison to the cornea, the sclera is the principle load bearing tissue of the eye (Norman et al., 2010) and its greater malleability, ensures that it responds to pressure from intra and extraocular muscle movements denoting its sensitivity to pressure change (Asejczyk-Widlicka & Pierscionek, 2008). As a dynamic structure, the sclera is able to substantially alter its shape and behavior in response to IOP changes (Quigley and Cone, 2013). The rheological property of elasticity that is defined as the ratio of stress (applied force) to strain (change in shape in response to applied force) is 3.0 to 3.5 times higher in the sclera than in the cornea (Asejczyk-Widlicka & Pierscionek, 2008). A study conducted by Pierscionek et al. (2007) demonstrated that the sclera deformed linearly to changes in IOP when tested in enucleated porcine eyes, whose scleral rigidity is very close to that found in human eyes (Olsen, 2002). Corneal curvature did not change whereas scleral curvature altered with incremental
increases in IOP between 15 mmHg and 50 mmHg, with increases at the rate of 0.1 mm/mmHg (Pierscionek et al., 2007). This is indicative that the sclera is the most sensitive ocular structure in terms of its response to IOP variations. Previous studies have also suggested that scleral IOP measurements are clinically useful (Khan et al., 1991; Frantz et al., 1994). This may result in less discomfort for the patient, especially after extended periods of time and overnight. Using the sclera would also prevent any obstruction of vision that may be more apparent with corneal monitoring devices. Regardless, the sclera has more surface area than the cornea allowing for more leverage when it comes to designing a strain gauge device embedded in a carrier material. The linear change in scleral curvature with changing IOP denotes that it can be a novel method for monitoring and measuring IOP over extended periods of time (Pierscionek et al., 2007).

In summary, the material properties of the sclera and its response to elevations in IOP render it as an obvious choice for the placement of a continuous IOP monitoring device. The assessment of the mechanical responsiveness of the sclera to IOP prove that it is a dynamic structure that alters its shape and behavior to IOP change. Biomechanical models (Sigal et al., 2011; Sigal et al., 2005) suggest that the behavior of the optic nerve head is strongly dependent on the biomechanical properties of the peripapillary sclera.
1.6 Thesis Organization

This thesis is organized in a multiple paper format in which chapters contain peer-reviewed documents that have been slightly altered for consistency of style. By following this structure, rather than a traditional thesis format, it allows the reader to further understand the conceptualization of the idea of a scleral strain gauge sensor for present and future use. Each chapter focuses on the completion of a specific aim for establishing the efficiency of a strain gauge to monitor IOP. I hope that it best answers the questions and concerns for future directions and permits consistency among the chapters themselves.

Chapter 1 serves as a general introduction of glaucoma and the importance of IOP, providing relevant studies on IOP during sleep, with subsequent relevance to clinical and animal trials. The chapter also focuses on monitoring devices of the past and present provides specifications of the pre-wired strain gauge and rationale for a “scleral” mounted monitoring device. The final section of this chapter serves to provide the scope of motivation for this project as well as the research aims and hypothesis for the PhD thesis.

Chapter 2 presents original research introducing a novel approach to monitoring IOP from the scleral surface. It addresses the application of the strain gauge to monitor IOP for temporary continuous use from scleral and conjunctival tissue in enucleated porcine eyes.

Chapter 3 presents original research and addresses the efficiency of using the strain gauge sensor in a live porcine model under anesthesia.

Chapter 4 presents original research assessing the use of a contact lens as a possible carrier material for the strain gauge and the inflammatory affect, if any, that it would have on the conjunctival surface in comparison to the corneal surface. By comparing the conjunctival and corneal surface, this dissertation provides a proof-of-principle on why the sclera may be a novel on-corneal alternative to IOP monitoring.

Chapter 5 contains the general discussion, concluding summary and emphasizes the potential for this technology in the clinical field.
1.7 Rationale, Aims and Hypothesis

This thesis is focused on providing proof-of-principle for a novel, scleral mounted device for measuring IOP continuously. The research work encompasses the fabrication of an IOP transducer apparatus and the characterization and analysis of a strain gauge sensor to monitor IOP over extended periods of time. The methodology was based on a non-corneal approach of analyzing strain gauge displacement via deformation of the scleral curvature due to increases in IOP as an indirect continuous monitoring form. Can a strain gauge sensor record IOP manipulations from the sclera? Would these recordings correlate to true IOP? The **global hypothesis** of this thesis is that the scleral surface mounted, strain gauge device will be able to accurately and safely measure changes in IOP over an extended period of time, providing a more sensitive approach to detecting small deformations caused by pressure manipulations. In general, such a device would revolutionize glaucoma care by providing a complete IOP profile aiding in the diagnosis and assessment of therapy for mild to moderately advanced glaucoma patients.

**Specific Aim 1:** To establish proof-of-concept that a pre-wired, commercially available, strain gauge sensor can measure manipulations in IOP in enucleated porcine eyes on the scleral and conjunctival tissue. (see Chapter 2).

**Specific Aim 2:** To establish proof-of-concept in a live animal model that a pre-wired strain gauge is able to recognize changes in IOP. The sensor will be wired to a nearby computer device and will be glued onto the surface of the sclera with 100% ethyl cyanoacrylate. The pig eyes will be subjected to changes in IOP by direct cannulation, while both strain and pressure readings will be recorded in order to capture the responses in strain, due to deformation of the sclera, in relation to changes in IOP (see Chapter 3).

**Specific Aim 3:** To assess the risk of wearing a silicone, hydrogel contact lens on the bulbar conjunctiva for an extended period of time and overnight as a plausible carrier for the strain gauge sensor. Risk assessment will be examined by flow cytometry of cells collected from the ocular surface epithelium. This aim was not to evaluate the behavior of the contact lens on the eye, but to examine the biocompatibility of a hydrogel contact lens on the scleral, bulbar conjunctiva during sleep in order to determine the material’s potential as a carrier for the proposed strain gauge sensor (see Chapter 4).
Chapter 2  Assessment of Pre-Wired Strain Gauge on Scleral and Conjunctival Tissue
2 Summary

This study aimed to determine the efficacy of measuring scleral deformation to indirectly measure intraocular pressure (IOP) change in porcine eyes. Fourteen porcine eyes were obtained within 48 hours post mortem from a local abattoir. A pre-wired strain gauge sensor (Omega Engineering, Inc., Stamford, CT) was mounted to the conjunctiva (n=8) and then directly to the sclera (n=6) in the superior temporal region of the eye. The anterior chamber was cannulated using a 27-gauge needle introduced from the temporal limbus and connected to a 30ml syringe filled with degassed water on a stand with variable height. Another 27-gauge needle was inserted into the anterior chamber through the nasal limbus primed with degassed water and connected to a pressure transducer. Adjusting the height of the syringe generated IOP changes and was recorded using a commercial software program (TracerDAQ™ Software, MicroDAQ.com Ltd., Contoocook, NH). IOP was increased in increments of approximately 15 mmHg from 0 to 44 mmHg (± 1.0 mmHg) and the surface deformation of the strain gauge sensor was recorded for each incremental step. IOP and surface deformation of the strain gauge sensor increased linearly with increasing volume. Analysis of variance gave a significant difference between strain gauge deformation and IOP manipulations (p<0.0001) with rho values ranging from 0.800 to 0.999 when mounted directly to the conjunctiva and 0.941 to 0.998 when mounted directly to the sclera. Changes in IOP can be accurately assessed by measuring changes in the radius of curvature of the sclera. These results provide a proof-of-concept for the realization of a conjunctiva/scleral-mounted sensor to monitor IOP. The ability of a strain gauge sensor to determine scleral deformation could be a novel, non-corneal approach to the measurement of 24-hour IOP.
2.1 Introduction

Intraocular pressure (IOP) follows circadian variations and fluctuations throughout a 24-hour period that is influenced by physiological factors, such as exercise, disease and drugs (Hughes et al., 2003; Liu, 1997; Sit 2009a). Recognized as the most important modifiable risk factor for the development and progression of glaucoma (Hughes et al., 2003; Mansouri & Shaarawy, 2011; Singh & Shrivastava, 2009; Sit, 2009a), the lowering of IOP levels by medical and surgical intervention is the only option available to reduce the development of blindness caused by this disease (Bhorade et al., 2009; Heijl et al, 2002; Kakaday et al., 2009). Current standards for glaucoma management involve single IOP readings during office hours, which provide an incomplete description of a patient’s IOP profile and its variability, particularly overnight. A retrospective study conducted by Mosaed et al. (2005) correlated office and peak nocturnal IOP in both healthy individuals and patients with glaucoma and found that 67 percent of the peak 24-hour IOP values in patients with glaucoma occurred at night. In addition, Hughes et al. (2003) demonstrated that peak IOP values in approximately 52 percent of patients occurred outside normal office hours and that 24-hour IOP monitoring led to a change in clinical management of 79 percent of patients with glaucoma. The reason for this finding is assumed to be an increase in episcleral venous pressure and the redistribution of body fluids. Several published reports have examined the variability of IOP and have concluded that a clearer understanding and continuous assessment of IOP variation throughout the 24-hour period would be beneficial for the diagnosis and management of patients with glaucoma (Bhorade et al., 2009; Mansouri & Shaarawy, 2011; Singh & Shrivastava, 2009; Sit, 2009a).

Current tonometric devices include applanation techniques (e.g. Goldmann and Perkins), pneumotonometry (e.g. Tonopen), dynamic contour and noncontact tonometry. These devices are unable to measure IOP over time and only provide estimates that depend on various factors for accurate measurements (Bhan et al., 2002; Ghaboussi et al., 2009). The GAT considered the gold standard, is dependent on corneal thickness, corneal curvature, corneal structure and axial length, overestimating IOP for individuals with thicker corneas and underestimating for individuals with thinner corneas (Asejczyk-Widlicka & Piersciencek, 2008; Fogagnolo et al., 2006; Nosch et al., 2010; Singh & Shrivastava, 2009). Although this method is well established in the clinical setting, the need for more frequent IOP measurements throughout the 24-hour period is pertinent.
period, independent of corneal anatomy and physiology, is needed for the better management of patients with glaucoma since a one-time IOP measurement is likely to be misleading providing a false diagnostic (Parikh et al., 2008). Several attempts have been made to establish such a clinical device, but have warranted no success (Cooper et al., 1974; Green and Gilman, 1974). Recently, Leonardi et al. (2004) developed a strain gauge sensor embedded in a wireless contact lens that measures deformation of the cornea associated with IOP changes. The device was based on the assumption that central corneal radius of curvature changes by approximately 3µm for every 1mHg change in IOP in enucleated pig eyes (Leonardi et al., 2004; Leonardi et al., 2009). Mansouri and Shaarawy (2011) presented results of a 24-hour human trial using a wireless version of the device, Triggerfish® (SENSIMED, Switzerland), in 13 patients with open-angle glaucoma. The authors reported the ability to monitor IOP fluctuations in patients over 24-hours with no adverse reactions other than keratitis in four patients (31 percent). There have been several recent reports validating the technique but large clinical trials remain to be performed. In addition there is no way to correlate between an individuals change in corneal curvature and the magnitude of change in IOP and all measurements remain in arbitrary units. Moreover, because the strain gauge is embedded in a silicon contact lens it would be subject to significant motion artifacts unless shaped to fit each individual’s cornea, a concept that was first introduced by Greene and Gilman (1974) when they proposed their soft contact lens IOP monitoring device some 30 years ago. Clearly this device, which relies on the correlation between IOP and corneal deformation, is subject to artifact due to inter-individual and intra-individual variation.

Pierscionek et al. (2007) conducted a study to determine the effect of changing IOP on the corneal and scleral curvatures in enucleated porcine eyes. The study illustrated that elastic moduli of the cornea and sclera are independent of IOP but that of the sclera is higher than that of the cornea. Thus, an increase in IOP alters the curvature of the sclera more than that of the cornea because of its biomechanical properties (Pierscionek et al., 2007). They also noted that the porcine eye is extremely similar to the human eye in terms of scleral rigidity. The study concluded that scleral curvature may be a novel approach to measuring IOP. There is a demand to monitor IOP fluctuations throughout the 24-hour period for diagnostic and management purposes. As a result, improved diagnostic tools need to be developed that are non-invasive, easy to use, are independent of corneal parameters and that measure IOP more frequently throughout the day (Liang et al., 2009).
To date, there remain no commercially available non-invasive devices for use in humans that can reliably and accurately measure IOP throughout the 24-hour period. The research objective was to demonstrate the efficacy of a noninvasive strain gauge sensor, placed on the sclera and conjunctiva, to detect changes in IOP. I hypothesized that a scleral mounted, strain gauge device will be able to accurately and safely measure changes in IOP over a 24-hour period, and aid in the diagnosis and management of patients with glaucoma. The specific aims are to provide proof-of-principle for a novel, scleral mounted device for measuring IOP and to establish whether IOP can be recorded from the sclera using enucleated porcine eyes.

The purpose of this study was to demonstrate that a strain gauge sensor, attached to the conjunctiva overlying the sclera, can reliably detect changes in IOP in enucleated porcine eyes. The ability of a strain gauge sensor to determine scleral deformation could lead to a novel, non-corneal approach to the measurement of 24-hour IOP.

2.2 Materials and Methods

2.2.1 Experimental Design and Procedures

Preliminary Enucleated Human Eye Experiments: Initial experiments were conducted on two donor human eyes obtained from the Canadian Eye Bank. These trials allowed for proper establishment of wiring and the ability to distinguish whether the transducer was working correctly in identifying IOP manipulations that were manually controlled. The human donor eyes (Eye Bank #128; #142) were set up in a 6-well, flat bottom, multiwall plate (Becton-Dickson, Mississauga, Canada; Lot #356515). This allowed for proper anchoring of the eyes, which aided with placement and gluing of the strain gauge. Each eye was gently washed with saline and then dried with a surgical sponge before placement of the strain gauge. Ethyl-2-cyanoacrylate, or Krazy glue (Scarborough, Canada; Lot #B0728), was used to bond the strain gauge to the bulbar conjunctiva. The cyanoacrylate glue hardens very quickly when trapped between two surfaces. The reaction is caused by the hydroxyl ions in water of the water vapor on surfaces that comes from the surrounding air. Thus, air humidity may alter the bonding affect of the glue. The curing reaction begins at the surface of a bonded material and moves towards the center of the bond. Thick applications of glue were avoided because they would harden less than surface to surface
bonds, causing polymerization reactions to occur before reaching the center of the bond. Thick applications of glue would also take longer to cure. One strain gauge measuring 9.4 by 2.8 mm, encapsulated with two lead wires, 3m long (OMEGA Engineering Inc. Stamford, Connecticut, KFG-5-120-C1-11L1M2R) was glued to each human donor eye 1mm from the limbus in the temporal, inferior region. Both eyes were cannulated with a 27G needle and connected to silicone tubing (Baxter, Lot#2C5645) that directly attached to the transducer. Wiring of the data acquisition module was made to the transducer as well as to the strain gauge. IOP manipulations were correlated to transducer readings obtained from the data acquisition module as well as the Tonopen.

Enucleated Porcine Eye Experiments: Experimentation continued using porcine eyes, due to their similarity in size and form to human eyes (Jantzen et al., 1992), and the desire to progress to live pig experiments. Only one strain gauge per porcine eye was employed to record the strain gauge response in these initial experiments. This allowed for the monitoring of any thermal drift, if at all, that would be experienced with a quarter-bridge circuit. Glycerin or saline were used to keep all eyes moist and to prevent them from drying out during experimentation. Experimentation with the enucleated porcine eyes also enabled to determine proper anatomical location for the strain gauge placement.

Once the transducer as well as for the wiring for the strain gauge was set up and properly recording with the data acquisition module, fourteen more enucleated porcine eyes were obtained from a local abattoir (Junction Meats, Toronto, ON) and used within 24-hours for testing. The eyes were placed within a custom holding apparatus and any excess ocular fat was trimmed, making sure that the upper and lower lids were intact as a way of helping to hold the strain gauge sensor in place (Figure 2-1). Two strain gauges bonded together were then glued to the superior,
region of the anterior sclera and/or bulbar conjunctiva with ethyl-2- cyanoacrylate. A half-bridge circuit was employed for these experiments. The experiments were carried out at room temperature. Eyes were kept moist during experimental set-up with the use of saline.

Six enucleated porcine eyes had the topmost layer of the conjunctiva removed in the area where the strain gauge was to be placed, approximately 1-2 mm from the limbus, superior temporally. Two strain gauges, firmly glued together, measuring 9.4 by 2.8 mm, encapsulated with two lead wires, 3m long (OMEGA Engineering Inc. Stamford, Connecticut, KFG-5-120-C1-11L1M2R) were used in order to compensate for thermal drift. By combining two strain gauges, a half bridge circuit was employed allowing for temperature changes to be compensated. Once the strain gauges were glued together, a surgical sponge was used to gently dry the cleaned area of the sclera before their attachment. The strain gauges were mounted to the sclera with the leads positioned face up. Tests were repeated serially for three sessions on the same eye with the same strain gauges attached in order to access repeatability. The remaining eight eyes had the strain gauge glued directly to the conjunctiva overlying the sclera, but in the same superior temporal location. Once the strain gauge was secured to the sclera or conjunctiva, the anterior portion of the cornea was then cannulated with a 27-gauge needle introduced from the nasal limbus and connected to a 20ml syringe filled with saline extending to a reservoir with adjustable height (Figure 2-2). A 23-gauge needle was inserted into the anterior chamber through the temporal limbus primed with degassed water and connected to a pressure transducer. IOP changes were generated by adjusting the height of the reservoir and recorded using a commercial software.
program (TracerDAQ™ Software, MicroDAQ.com Ltd., Contoocook, NH) (Figure 2-3). Active recordings were set for five minutes with 15-minute intervals between rest periods and IOP adjustments. Indirect IOP recordings were measured using a Tono-Pen XL to decipher a correlation between the IOP transducer apparatus and the Tonopen-XL. The Tonopen-XL was used because of its portability.

Figure 2-2. Photographs of Enucleated Porcine Eyes Obtained From A Local Abattoir. Used within 24-hours for IOP experimentation. Each porcine eye was cannulated with a 27-gauge needle inserted into the anterior chamber and connected with a silicone tube to a column filled with saline attached to a pressure transducer via a stopcock. Another 27-gauge needle attached to a reservoir of saline on a stand of variable height is also inserted into the anterior chamber and is used to control the IOP. The strain gauge is secured to the sclera with 100% ethyl 2-cyanoacrylate and the upper and lower lids were left intact (A,B,C,E,F) to aid with holding the strain gauge in place after gluing. Having the lids intact helped with keeping the eye moist. Under certain circumstances, the porcine eyes had to be kept moist by being placed on a moist Kim wipe to prevent from drying out because of the loss of the eyelid tissue (D).
Figure 2-3. Schematic of the Enucleated Porcine Eye Model. The pig eyes are cannulated with a 27-gauge needle inserted into the anterior chamber and connected with a silicone tube to a column filled with saline attached to a pressure transducer via a stopcock. Another 27-gauge needle attached to either a syringe pump or a reservoir of degassed water on a stand of variable height is also inserted into the anterior chamber and is used to control the IOP. The pre-wired strain gauges are connected into a Wheatstone bridge circuit with a combination of two active gauges (half bridge). The Wheatstone bridge configuration compensates for thermal drift. An amplifier is also connected to increase sensitivity. The strain gauges are secured to the sclera or conjunctiva with 100 percent ethyl 2-cyanoacrylate.

2.2.2 Strain Gauge Sensor

Two strain gauges were attached to each other to double sensitivity, forming a half-bridge circuit and were exercised prior to taking readings for about 15 minutes to establish connectivity. An amplifier was used to power the pressure transducer and to filter and amplify the output. A Wheatstone bridge configuration was used to allow for an increase in sensitivity and help compensate for thermal drift. The pressure in the eye was adjusted from 0 mmHg to 52 mmHg and again from 52 mmHg to 0 mmHg in steps of 7 to 14 mmHg and each pressure level was held for approximately 60 seconds. The range of IOP increments was chosen to represent a variation...
of IOP that are present in the clinical setting (Leonardi et al., 2009; Van der Valk et al., 2005). Each recording period lasted 5 minutes with 30,000 data points collected for post-analysis. Strain gauge signals have been corrected by subtracting the thermal response that was measured in the original state of the strain gauge when not adhered to the ocular surface. The strain gauge sensor was not zeroed for each experiment, but the difference between the initial response and the response obtained once the IOP was adjusted was used as a baseline measurement for each individual experiment. For The graph obtained from the IOP changes (transducer) was compared to the graph obtained from the changes in the strain gauge.

2.2.3 Data Analysis

Data were analyzed by linear regression, to assess the relationship between changes in IOP and the deformation of the strain gauge, using Prism™. The coefficient of determination of linear regression, $r^2$, was also calculated to determine the correlation between the two units. Data are reported as mean values $\pm$ standard deviation. Statistical significance was set as a p-value of $\leq 0.050$ and $r^2$ (rho) values of 0.800 or greater.

2.3 Results

2.3.1 Preliminary Enucleated Human Eye Results

Two human donor eyes received from the Canadian Eye Bank were used for trial-run experiments to determine connectivity of the strain gauge and transducer apparatus. Human donor eye #128 was enucleated from a 53 year-old female, 24-hours after post-mortem, whose cause of death was listed as unknown (sepsis assumed). Human donor eye #142 was enucleated from a 57 year-old male, 24-hours after post-mortem, whose cause of death was listed as aneurysm. A single strain gauge was adhered to the bulbar conjunctiva and IOP manipulations from 0 mmHg to 60 mmHg were recorded. Connectivity of strain gauge and transducer apparatus was well established and showed good correlation between IOP recordings and level at which the height of the reservoir was adjusted. The strain gauge also correlated well to the manual IOP changes, resulting in a linear regression of $r^2=0.948$. The experiment was then repeated with the conjunctiva gently removed, exposing the anterior sclera. Figure 2-4 illustrates the results from human donor eye #128 with the strain gauge attached to the conjunctiva. The
figure (2.4) shows the response obtained from the transducer, the strain gauge, thermal drift correction with a quarter-bridge circuit and baselines of the strain gauge attached to the conjunctiva and non-attached to the ocular surface. The sample count was averaged from 30,000 data points to 300 over a five-minute period.

**Figure 2-4. Strain Gauge Deformation from IOP Changes in a Human Donor Eye When Placed on the Bulbar Conjunctiva.** (A) Plot representation of transducer voltage versus strain gauge voltage in human donor eye (#128). The strain gauge was attached to the bulbar conjunctiva with ethyl-2-cyanoacrylate. (B) Linear regression of the strain gauge average corrected for thermal drift shows a good correlation of \( r^2 = 0.948 \). A slight thermal drift of 0.05V is apparent and corrected for. Recording was performed for a five-minute period with the collection of 30,000 data points that were averaged over a 300-sample count.
Figure 2-5 represents the same human donor eye (#128) with the conjunctiva gently removed and the experimental procedure repeated. The linear regression of the strain gauge corrected for thermal drift showed a good correlation of $r^2=0.996$ when it was applied directly to the sclera.

**Figure 2-5. Strain Gauge Deformation from IOP Changes in a Human Donor Eye When Placed Directly on the Sclera.** (A) Plot representation of transducer voltage versus strain gauge voltage in human donor eye (#128). The strain gauge was attached directly to the sclera with ethyl-2-cyanoacrylate. (B) Linear regression of the strain gauge average corrected for thermal drift shows a good correlation of $r^2=0.996$. 

$y = 7.10 \times 10^{-5} x + 7.18 \times 10^{-2}$

$R^2 = 0.996$
2.3.2 Enucleated Porcine Eye Results

Trial-run experiments were also performed with enucleated porcine eyes (n=3). Figure 2-6 (Eye 3OD), 2-7 (Eye4OS) and 2-8 (Eye5OS) illustrate the profile of strain gauge responses to IOP manipulations with strain gauge voltage corrected for thermal drift of enucleated porcine eyes with the conjunctiva removed. The strain gauge was directly attached to the sclera and recordings were averaged to 300 samples from 30,000 data points over a five-minute period.

![Figure 2-6. Profile of Enucleated Porcine Eye with Strain Gauge Bonded to Sclera with Ethyl-2-cyanoacrylate.](image)

Measurements for strain gauge baseline (SGVbaseline), strain gauge, strain gauge thermal drift correction (SGVdriftcorrected) and IOP transducer were recorded over a five minute period. Strain gauge baseline was recorded for a five minute period, free of any attachment to the ocular surface. The baseline clearly shows a drift of 0.006V over the recorded time period. The strain gauge was corrected for thermal drift by subtracting the difference from the baseline recording.
Testing was performed with the removal of the conjunctiva and the strain gauge being placed directly on the scleral surface. Two baseline measurements were performed (SGVbaseline 1; SGVbaseline 2). Baseline recordings involve the recording of the strain gauge being placed on a flat surface. Ideally, the strain gauge baseline should provide a straight line, if thermal drift would be negated. SGVbaseline 1 demonstrated an extreme thermal drift response and was thus repeated. The strain gauge response showed no correlation whatsoever with the IOP changes, indicative that either the connection or the set-up was not properly initiated.
Figure 2-8. Profile of Strain Gauge Deformation with IOP Changes in a Porcine Eye. Although the strain gauge response was inverted, most likely due to the wiring of the channels, the overall response of the strain gauge to IOP manipulations was linearly correlated. The baseline recording of the strain gauge showed a slight effect of thermal drift of 0.001 V, quite minimal for a quarter-bridge circuit. Recordings were performed over a five-minute period. IOP increments ranged from 0mmHg to 60mmHg. Data points were averaged to correspond to 600 samples.

Figure 2-8 (Eye5OS) of the trial-run porcine eyes, showed the best correlation of strain gauge displacement to IOP manipulations. A minimal thermal drift of 0.001 V was prevalent as indicative from the baseline recording. Figure 2-9 displays the linear regression of the transducer and strain gauge voltages for the enucleated porcine eye (Eye5OS). Recordings were performed twice with linear regression being $r^2=0.987$ for the first recording and $r^2=0.984$ for the second recording. The second recording was time adjusted for comparison between recordings. Figure 2-10 illustrates different measurements of strain gauge baselines under varying conditions. The strain gauge was measured at baseline for a five-minute period. The baseline recording shows no indication of thermal drift and is linear for the entire recording time. The strain gauge was recorded several times while being mounted to the ocular surface without any pressure...
inferences. The figure illustrates the thermal drift of the strain gauge as a result of the resistance caused by the expansion of the sclera in response to IOP changes when it is mounted.

Figure 2-9. Transducer Versus Strain Gauge Displacement (mV). Eye5OS showed high linearity of IOP manipulations and deformation of strain gauge. Test 1 resulted in a rho value of 0.987 and test 2 resulted in a rho value of 0.984. The difference in linear regression could be due to the time required for the ocular tissue to accommodate back to an equilibrium state of rest where no pressure was induced. Testing was repeated to ensure accuracy. Test 2 was time adjusted to compare to initial testing.
Strain gages were recorded in variable states that included (1) unmounted with no pressure induced (baseline); (2) mounted with slight pressure adjustments done by palpitation of the finger; and (3) mounted with no pressure induced. The figure illustrates how temperature may affect the strain gauge measurement accuracy when mounted on to the test specimen. Baseline recording was ideal, but slight drifts can be documented once the strain gauges are mounted onto the scleral surface.

### 2.3.3 Thermal Drift Experiments

Figure 2-11 depicts the thermal drift of the strain gauge when mounted on Eye5OS with no changes in pressure applied. The strain gauge deforms as a result of the resistance caused by the thermal output. It should be noted that the trial-run experiments were not tested with the use of a half-bridge circuit, but rather a quarter-bridge circuit. A half-bridge circuit is intended to minimize sensitivity to temperature by processing the gauge material to compensate for the thermal expansion of the specimen material for which the gauge is intended. The thermal drift is in certain instances produced a large error in static measurements as was noted for the baseline recording in Figure 2-7.
Changes in temperature that could affect the reading output for the strain gauge may be a result of changes in ambient pressure or application of saline to ocular tissues. Since the bonded strain gauge is a passive rather than active sensor, an excitation current passes through the strain gauge to output an electrical signal. This causes the strain gauge to heat up. Any addition to the ocular surface with the strain gauge attached that would render a cooling effect, such as saline, may cause an error reading because of the self-heating effect. Moreover, improper gluing of the strain gauge, such as placing too much glue or not adhering the strain gauge surface-to-surface, causing apparent voids to the test specimen, may render errors in recordings.
Experiments were performed to determine the temperature shift of the strain gauge sensor at various temperatures. This was evaluated by immersing the strain gauge in a water bath filled with water and measuring the Wheatstone bridge output signal (Figure 2-12). The temperature of the water bath was increased from 20°C to 44°C and the output signal was recorded for 15 seconds at each temperature increment.

![Figure 2-12. Thermal Drift Analysis Of Strain Gauge Sensor.](image)

**Figure 2-12. Thermal Drift Analysis Of Strain Gauge Sensor.** Measurement of the thermal drift of the strain gauge in a water bath with temperatures ranging from 21 to 41 degrees Celsius. The strain gauge sensor demonstrates high temperature stability at temperatures ranging from 31 to 41 degrees Celsius.

**Table 2-1. Pre-Wired Strain Gauge Specifications.**

<table>
<thead>
<tr>
<th>Specification</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bridge supply Voltage (Vo)</td>
<td>5.0V</td>
</tr>
<tr>
<td>Resistance value of gauge</td>
<td>120 Ohms</td>
</tr>
<tr>
<td>Strain gauge material</td>
<td>Constantan foil</td>
</tr>
<tr>
<td>Gauge factor (GF)</td>
<td>2.08 ± 1.0%</td>
</tr>
<tr>
<td>Sensitivity (Live Pig Experiment)</td>
<td>0.569mV/mmHg</td>
</tr>
<tr>
<td>Thermal drift</td>
<td>0.01mV/°C</td>
</tr>
</tbody>
</table>
It should be noted that the eye’s temperature is stable around 34°C (Efron et al., 1989), but that changes in atmospheric temperature or changes in the ocular surface (i.e. eye drops) may cause variations in the signal. The thermal drift of the strain gauge in a water bath indicated that a 1mmHg IOP change corresponds to 0.569mV (live pig experiments-Chapter 3) (Table 2-1) that would relate to a temperature change of 0.01 mV/°C. As shown in Figure 2-12, the thermal drift of the strain gauge at 21 to 30 ºCelsius was 1 mV/°C and at 31 to 40 ºCelsius was 0.1 mV/°C is low. Additionally, a Wheatstone bridge configuration was applied in order to compensate for thermal drift and to allow for the measurement of small resistance changes or minute strains. This was accomplished by forming a half bridge circuit where the output voltage of the bridge is doubled. The limitation of the use of a half bridge is the requirement of finding the same but opposite strain. Thus, two strain gauges were glued to each other and then applied to the tissue.

2.3.4 Calibration Experiments

The strain gauge was then exercised through a series of experiments to test calibration and response to thermal drift with a half-bridge configuration. Two strain gauges glued together forming the strain gauge sensor were then glued to a pipette aid that was manually manipulated to deform the sensor. A time series of experiments were conducted where the strain gauge was manipulated to produce an output signal (Figure 2-13). The strain gauge correlated well to pressure changes that were manually induced by the pipette aid. A slight drift can be seen at baseline that may have been caused as a result of leakage from the pipette aid. Yet, the correlation between the pressure sensor transducer and the strain gauge displays good correlation. The manipulations were repeated three times and resulted in an output of approximately 0.5mV per mmHg from a half bridge configuration. Hence, this is one reason why it was opted to use two strain gauges glued together forming a half bridge circuit to negate the affect of the resistance of the strain gauge to changes in pressure. A transducer was fabricated to measure pressure inside the eye via cannulation of the anterior chamber, representing true IOP. A full schematic of the transducer apparatus is shown in Figure 2-3. The transducer apparatus was calibrated and a profile of pressure manipulations in incremental steps from 0 to 68 cmH₂O was performed to access the transducer function (Figure 2-14). Figure 2-14 shows a profile of the incremental pressure changes and good correlation to manual pressure adjustments with standard deviation.
Figure 2-13. Calibration Of Strain Gauge Sensor With Half-Bridge Circuit. Half bridge calibration of the strain gauge sensor on a pipette aid showing repeat profiles and their correlation with standard deviations. Slight drift is observed for baseline measurement, but may be due to recording error or technical error (i.e. gluing to pipette aid). All calibrations show good linearity with transducer.
Figure 2-14, Correlation Of Transducer To IOP Manipulations. (A) Profile of transducer output to IOP manipulations caused by alterations to the level of the reservoir of saline, inducing pressure changes. IOP was increased from 0 cmH\textsubscript{2}O to 70 cmH\textsubscript{2}O and decreased from 70 cmH\textsubscript{2}O to 0 cmH\textsubscript{2}O in increments of 10 cmH\textsubscript{2}O. (B) Illustrates good linearity of IOP alterations manually controlled to transducer output signal obtained via the data acquisition module. Rho value of 0.999 and standard deviations as small as 0.001 show good linearity.

In all circumstances, IOP variation was correlated linearly with strain gauge deformation resulting in acceptable rho values. The enucleated porcine eyes without the conjunctiva, the pressure in the eye was inflated from 0 mmHg to 52 mmHg in steps of 7 mmHg and deflated in the same manner from 52 mmHg to 0 mmHg in steps of 7 mmHg. All enucleated porcine eyes tested without the conjunctiva had a rho value in the range of 0.941 to 0.991 with increasing IOP and 0.912 to 0.998 with decreasing IOP (Table 2-2). Figure 2-15(A-F) shows the profiles of the strain gauges’ displacement versus the IOP variation and the sample count over a five-minute period with the correlation of the voltage versus pressure with standard deviations. Of the 30,000 data points collected, the sample count was averaged to give 600 data points in order to reduce the signal to noise ratio. All linear regression coefficients were above \( r^2 = 0.800 \). The assumption of linearity of the data was supported by analysis of variance (\( p < 0.0001 \)). The minimum sensitivity observed in the six enucleated eyes was 0.440 mV/mmHg and the maximum was 2.50 mV/mmHg with increasing IOP; 0.590 mV/mmHg minimum and 2.50 mV/mmHg maximum with decreasing IOP. The mean sensitivity was 1.04 mV/mmHg with a standard deviation of \( \pm 0.772 \) mV/mmHg with increasing IOP. The mean sensitivity was 1.23 mV/mmHg with a standard deviation of \( \pm 0.677 \) mV/mmHg with decreasing IOP. The enucleated porcine eyes with
the conjunctiva left intact yielded rho values in the range of 0.800 to 0.999 with increasing IOP and 0.870 to 0.999 with decreasing IOP.
A.

B.
C.

![Graph showing strain gauge output versus pressure (mmHg). The graph includes two lines, one for increasing pressure (0-52 mmHg) and one for decreasing pressure (52-0 mmHg). The equations and correlation coefficients for the lines are provided: for increasing pressure, \( y = 6.21E^{-4}x + 1.61E^{-2} \) with \( r^2 = 0.968 \); for decreasing pressure, \( y = 9.22E^{-4}x - 6.79E^{-4} \) with \( r^2 = 0.977 \).]

D.

![Graph showing strain gauge output versus pressure (mmHg). The graph includes two lines, one for increasing pressure (0-52 mmHg) and one for decreasing pressure (52-0 mmHg). The equations and correlation coefficients for the lines are provided: for increasing pressure, \( y = 1.34E^{-3}x + 1.71E^{-3} \) with \( r^2 = 0.991 \); for decreasing pressure, \( y = 1.43E^{-3}x - 2.87E^{-3} \) with \( r^2 = 0.993 \).]
Figure 2-15. Recordings of strain gauge sensor in response to IOP changes when applied to the sclera in enucleated porcine eyes (N=6). (A-F) Profiles of the strain gauges’ displacement versus the IOP variation and the sample count over a five-minute period paired with the correlation of the voltage versus pressure with standard deviations (± SD) with the strain gauge glued directly on to the sclera. Red line - strain gauge; black line – transducer.
Figure 2-16(A-H) shows the profiles of the strain gauges’ displacement versus the IOP variation and pressure (mmHg) displayed with their correlation of averaged, corrected voltage versus pressure with standard deviation. The sample count was averaged to give 300 data points over a five-minute period. A thermal couple was placed directly on the strain gauge to measure changes in temperature during recordings. The temperature of the active strain gauge when not placed on the enucleated eye was 27.2°Celsius and 18.9°Celsius when glued to the conjunctiva. The strain gauge’s temperature ranged 1.4°Celsius from initial temperature reading and did not seem to reach temperatures above 21°Celsius when glued to the ocular surface. The baseline for the strain gauge was stable and a direct relationship was apparent with incremental increases in transducer voltage. The graphs emphasize a linear correlation in IOP variation and strain gauge deformation. The graphs demonstrate a stronger correlation with decreasing IOP than with increasing IOP. This may be due to fluidic resistance and again elastic properties of the conjunctiva. The minimum sensitivity observed in the eight enucleated eyes without the removal of the conjunctiva was 0.397 mV/mmHg with increasing IOP and 0.356 mV/mmHg with decreasing IOP. The maximum was 60.5 mV/mmHg with increasing IOP and 58.7 mV/mmHg with decreasing IOP. The mean sensitivity was 8.52 mV/mmHg with a standard deviation of 21.0 mV/mmHg with increasing IOP and 8.29 mV/mmHg with a standard deviation of 20.4 mV/mmHg with decreasing IOP.
Figure 2-16. Recordings Of Strain Gauge Sensor In Response To IOP Changes When Applied To The Conjunctiva In Enucleated Porcine Eyes (N=8). A-H shows the profiles of the strain gauges’ displacement versus the IOP variation and pressure (mmHg) with their correlation of averaged, corrected voltage versus pressure with standard deviation (± SD) in enucleated porcine eyes with strain gauge bonded directly to the glued conjunctiva with 100% ethyl-2-cyanoacrylate. Strain gauge deformations (red solid line) show good correlation to the transducer (black solid line).
Table 2-2. Rho Values For Increasing And Decreasing IOP Manipulations In Enucleated Porcine Eyes With The Conjunctiva Removed (Sclera) And With The Conjunctiva Left Intact.

<table>
<thead>
<tr>
<th>Sclera (n=6)</th>
<th>Increasing IOP</th>
<th>Decreasing IOP</th>
<th>Conjunctiva (n=8)</th>
<th>Increasing IOP</th>
<th>Decreasing IOP</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.981</td>
<td>0.998</td>
<td>A</td>
<td>0.828</td>
<td>0.870</td>
</tr>
<tr>
<td>B</td>
<td>0.956</td>
<td>0.989</td>
<td>B</td>
<td>0.930</td>
<td>0.950</td>
</tr>
<tr>
<td>C</td>
<td>0.968</td>
<td>0.977</td>
<td>C</td>
<td>0.921</td>
<td>0.935</td>
</tr>
<tr>
<td>D</td>
<td>0.991</td>
<td>0.993</td>
<td>D</td>
<td>0.857</td>
<td>0.965</td>
</tr>
<tr>
<td>E</td>
<td>0.960</td>
<td>0.996</td>
<td>E</td>
<td>0.800</td>
<td>0.916</td>
</tr>
<tr>
<td>F</td>
<td>0.941</td>
<td>0.912</td>
<td>F</td>
<td>0.999</td>
<td>0.999</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
<td></td>
<td>0.894</td>
<td>0.886</td>
</tr>
<tr>
<td>H</td>
<td></td>
<td></td>
<td></td>
<td>0.936</td>
<td>0.944</td>
</tr>
</tbody>
</table>

Actual IOP measurements by cannulation of the anterior chamber were compared against IOP estimates measured by tonometer (Tono-Pen XL). Tonometry measurements were taken three times before each recording at different IOP elevations. The analysis of the Tono-Pen XL and the measurements taken from the transducer, which are perceived to be true IOP, showed relatively high correlation as shown by linear regression ($r^2=0.971$; 0.984; 0.948). The assumption of linearity of the data was supported by analysis of variance ($p<0.0001$) (Figure 2-17). It should be noted that the Tono-Pen XL.
Sensitivity of the strain gauge, expressed quantitatively as ratio of fractional change in electrical resistance to the fractional change in length, was calculated for both scleral and conjunctival tissues (Table 2-3). The range of sensitivity for the strain gauge placed directly on the scleral tissue was 0.493 to 2.50 mV/mmHg with increasing IOP and 0.590 to 2.50 mV/mmHg with decreasing IOP. The range of sensitivity for the strain gauge placed on the conjunctiva was 0.397 to 60.5 mV/mmHg with increasing IOP and 0.356 to 58.7 mV/mmHg with decreasing IOP.
Table 2-3. Sensitivity Values of the Strain Gauge When Placed on the Sclera and the Conjunctiva for Increasing and Decreasing IOP

<table>
<thead>
<tr>
<th>ID</th>
<th>Sclera Sensitivity Increasing IOP (mV/mmHg)</th>
<th>Conjunctiva Sensitivity Increasing IOP (mV/mmHg)</th>
<th>Sclera Sensitivity Decreasing IOP (mV/mmHg)</th>
<th>Conjunctiva Sensitivity Decreasing IOP (mV/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.655</td>
<td>0.605</td>
<td>0.590</td>
<td>0.635</td>
</tr>
<tr>
<td>B</td>
<td>0.809</td>
<td>1.39</td>
<td>1.06</td>
<td>1.39</td>
</tr>
<tr>
<td>C</td>
<td>0.621</td>
<td>0.480</td>
<td>0.922</td>
<td>0.478</td>
</tr>
<tr>
<td>D</td>
<td>1.34</td>
<td>0.397</td>
<td>1.43</td>
<td>0.356</td>
</tr>
<tr>
<td>E</td>
<td>0.493</td>
<td>0.478</td>
<td>0.893</td>
<td>0.357</td>
</tr>
<tr>
<td>F</td>
<td>2.50</td>
<td>60.5</td>
<td>2.50</td>
<td>58.7</td>
</tr>
<tr>
<td>G</td>
<td>-</td>
<td>1.80</td>
<td>-</td>
<td>1.62</td>
</tr>
<tr>
<td>H</td>
<td>-</td>
<td>2.47</td>
<td>-</td>
<td>2.82</td>
</tr>
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2.4 Discussion

In this study we examined a non-corneal approach to measuring IOP, illustrating that changes in IOP can be accurately assessed by the degree of alterations in scleral radius via strain gauge dynamics. IOP and surface deformation of the strain gauge sensor increased linearly with increasing volume. Analysis of variance gave a significant difference between strain gauge deformation and IOP manipulations (p<0.0001) when mounted directly to the sclera and the conjunctiva. These results provide a proof-of-concept for the realization of a sensor to monitor IOP. The ability of a strain gauge sensor to determine conjunctival and/or scleral deformation could be a novel, non-corneal approach to monitoring IOP over an extended period of time.

Porcine eyes were chosen for experimentation because of their anatomical similarities with human eyes in terms of globe size, dimension and scleral rigidity (Pierscionek et al., 2007). The anterior chamber depth of a 40-year-old human (2.67 mm) is equivalent to that of a six-month old pig (2.67 mm) (Langner et al., 2010). Nonhuman primate animal models are expensive, limiting their usefulness for a detailed study of IOP. Other models, such as the rabbit, suffer from marked differences in anatomical features compared to humans. The pig is phylogenetically close to human and is much more available than the monkey. The pig eye shares many similarities with that of the human eye, both in terms of its structure and size. The pig retina is even more similar to the human retina than that of other large mammals such as the dog. Also, the pig has been recently used in ophthalmological studies to evaluate optical coherence tomography, corneal topography imaging, multifocal electroretinography and in models of retinitis pigmentosa, making this animal a good model for ophthalmological studies. Nevertheless, to be valid, an experimental model must provide IOP stability that is not affected by the cannulation of the anterior chamber for direct IOP measurements. This limits the usefulness of monkeys and rabbits for IOP studies, because in these species corneal puncture results in a breakdown of the blood-aqueous barrier producing protein exudation with subsequent glaucoma (Jantzen et al., 1992). The pig, apparently devoid of such vulnerability, is an established model in experimental ophthalmology, including studies of IOP. In short, the pig model allows us to closely mimic the clinical situation and monitor a device for IOP measurement since continuous IOP monitoring in glaucoma with high reliability is desirable for appropriate disease management.
Initial experimentation performed on human donor eyes (n=2) and enucleated porcine eyes (n=3) provided insight as trial and error runs for establishing proper wiring of the strain gauge and transducer and initial set-up of the following experiments. A quarter-bridge circuit was originally employed, causing variations in strain gauge response due to thermal drift. The circuitry was then changed to a half-bridge to compensate for temperature changes.

The difficulties incurred during testing included several factors. First, amount of glue being used to bond the strain gauge to the ocular tissue. If too much glue was employed, the strain gauge would stiffen and would be unable to properly bend or deform in response to the changes in IOP. Too little glue resulted in the strain gauge continually being shifted and not recording from a constant placement. Improved attachment methods or materials needed to be developed for proper adherence of strain gauge. Initially placement of the strain gauge was questioned. Where on the ocular surface would it be most comfortable to place a strain gauge for extended monitoring if this would be applied in humans? The temporal, superior region of the anterior sclera proved to be easily accessible for bondage of the strain gauge sensor. Second, fluid lines in the testing system were replaced with less rigid tubing, composed of silicone. This would ensure that pressure changes are directly influenced by the activities in the eye and not due to any compliance in the walls of the tubing. Third, a variety of small gauge needles were used during preliminary testing in an attempt to determine whether clogging of the fluid line, or in the needle, could be prevented. The 27G and 25G needles were often used for their ability to cause the least amount of problems associated with blockage. Smaller gauge needles were optimal when cannulating to prevent clogging of the fluid line and allow for easier delivery and withdrawal of fluid to and from the eye. Technically, cannulation of the anterior chamber, if not properly performed, would result in blockage of fluid or reduced pressure, resulting in false pressure readings.

There are several unique advantages of using a non-corneal approach to the continuous measurement of IOP. First, placing a strain gauge on the scleral surface works well with all eyes. Contact lens parameters do not enable a good or comfortable fit between the corneal contour and the contact lens surface in all eyes. Second, the eye is not exposed to the risk of an extended wear contact lens, particularly given the need for a tight fit with a silicon lens to minimize lens movement and the age of the typical patient with glaucoma. In addition the elderly glaucoma patient is unlikely to be an experienced contact lens wearer and will likely have a compromised
corneal surface and tear film due to dry eye and the effect of preservatives within their glaucoma medications, making them more prone to adverse events related to extended contact lens wear and lens removal. The sclera is the preferable placement for a strain gauge sensor because of its lower sensitivity and less susceptibility to injury than the cornea (Piso et al., 2012). Limitations of the scleral approach include the need to attach the sensor to the conjunctiva, a loose, gelatinous-like tissue. However, our results demonstrate that if sufficient surface area of conjunctiva is used the measurements are similar to those recorded directly form the scleral surface.

Over the years various attempts have been made to develop a device that can accurately measure 24-hour IOP in order to aid in the monitoring and management of glaucoma. Non-invasive as well as invasive devices have been introduced. The most challenging aspects of these devices are not only the location of where they will be placed in or on the eye, but most importantly their long-term biocompatibility. In 1974, Greene and Gilman introduced the first non-invasive method for monitoring IOP. Their system consisted of a soft contact lens with a strain gauge sensor that measured changes in IOP by sensing the deformation of the meridional angle. In 1979, Cooper et al. used a continual scleral guard ring applanation transensor to measure IOP in rabbits and dogs. In addition, Leonardi et al. (2004) employed a soft contact lens made of silicone with an embedded micro gauge to monitor IOP via change in corneal curvature. Yet, inter-individual and intra-individual variation in corneal thickness and rigidity, particularly in patients with corneal irregularities, could result in measurement error or deviations when relying on measuring IOP via corneal curvature (Browning et al., 2004; Doughty and Zaman, 2000). More recently, Leonardi et al. (2009) developed a wireless silicone contact less sensor for continuous monitoring of IOP. The device was adapted and tested on enucleated pig eyes and demonstrated minimal invasiveness and functionality in monitoring IOP. Yet, factors such as corneal hydration, contact lens induced stromal dehydration, keratometry and corneal diameter must be further investigated in a large-clinical trial. Other limitations of Leonardi’s contact lens device may include biocompatibility with prolonged use. Although the lens material is silicone based, a highly oxygen permeable material, prolonged placement of the sensor in a closed eye may compromise the epithelial barrier causing corneal swelling. Hence, Mansouri and Shaarawy who performed the first clinical trial using Leonardi’s wireless silicone contact lens noted no serious adverse events other than 31 percent punctate keratitis (Mansouri & Shaarawy, 2011).
Mansouri et al. (2012) also published another study examining the safety, tolerability and reproducibility of the contact lens sensor in patients with glaucoma. The study enrolled 40 patients suspected of having glaucoma (n=21) or with established glaucoma (n=19) and found that the recorded IOP patterns demonstrated good safety and tolerability with fair to good reproducibility. However, 82 percent of participants’ experienced blurred vision, 80 percent experienced hyperemia and 15 percent developed punctate keratitis (Mansouri et al., 2012).

There have been several attempts to find a practical and portable solution for IOP monitoring (Cooper et al., 1979; Greene & Gilman, 1974; Schnell et al., 1996), but to date no non-invasive devices have been developed that provide 24-hour monitoring of IOP from a non-corneal ocular surface in humans. The development of this device will be beneficial in understanding and managing IOP variations in glaucoma.
Chapter 3  Continuous Scleral Intraocular Pressure Monitoring in a Live Porcine Model
3 Summary

To assess the feasibility of continuous IOP monitoring from the bulbar conjunctival sclera in a live porcine model. Nine eyes from six Yorkshire strain pigs, 30-50 kg were used. A strain gauge sensor (Omega Engineering, Inc., Stamford, CT) was mounted with 100 percent cyanoacrylate to the conjunctiva approximately 1-2 mm from the limbal margin in the superior temporal region of the pig’s eye. The animals were anesthetized with isoflurane administered with 100 percent oxygen delivered through a facemask. Eyes were anesthetized with 0.5 percent proparacaine hydrochloride. The anterior chamber was cannulated using a 27-gauge needle introduced from the temporal limbus and connected to a 30ml syringe filled with degassed water on a stand with variable height. Another 27-gauge needle was inserted into the anterior chamber through the nasal limbus primed with degassed water and connected to a pressure transducer. Adjusting the height of the syringe generated IOP changes and was recorded with a commercial software program (TracerDAQ™ Software, MicroDAQ.com Ltd., Contoocook, NH). IOP was increased in increments of 7 mmHg from 0 to 44 mmHg (± 1.0 mmHg) and the surface deformation of the strain gauge sensor was recorded for each incremental step. IOP changes were repeated three times to establish reproducibility and linearity of the strain gauge sensor. IOP measurements and surface deformation of the strain gauge sensor responded linearly with changes in ocular pressure in a live pig model (n=9). Analysis of variance gave a significant difference between strain gauge deformation and IOP manipulations (p<0.005) with rho values ranging from 0.884 to 0.976 with increasing IOP and 0.808 and 0.983 with decreasing IOP. These proof-of-principle experiments are an essential step to the realization of a conjunctival/scleral mounted, 24-hour IOP recording device. Changes in IOP can be accurately assessed by measuring changes in the radius of curvature of the sclera in a live porcine model.
3.1 Introduction

The necessity to monitor IOP continuously over a period of time is desirable for the optimal assessment, diagnosis and treatment of individuals with glaucoma. Glaucoma is a group of neurodegenerative diseases associated with high IOP that may cause an optic neuropathy and excavation of the optic disc with a characteristic pattern of functional loss. The most common form of glaucoma is POAG; usually asymptomatic, it can develop with a higher than normal IOP in which the IOP increases over time but without any corneal edema or ocular pain present (Sharts-Hopko and Glynn-Milley, 2009). However, it can also develop with a normal IOP, in both cases it is likely that poor perfusion of the laminar cribrosa region of the anterior optic nerve is a contributory factor. The level of IOP that causes anatomical change is not the same in every eye, and some individuals may tolerate a pressure for an extended period of time that might cause vision loss in others. Regardless of biological variability within individuals, lowering IOP is still the only proven clinical treatment for halting the progression of the disease (Heijl et al., 2002; Crawford-Downs et al., 2011). By continuously monitoring IOP over extended periods of time, the ability to diagnose, treat and manage ambiguities that include patients with elevated IOP (ocular hypertension) who never develop glaucoma, and patients that develop glaucoma with no elevated IOP (NTG) will prove beneficial (Crawford-Downs et al., 2003).

Clinically, IOP is most frequently measured by applanation of the cornea at a single point in time, using Goldmann applanation tonometry (GAT). GAT is the current clinical standard, but is relatively imprecise with a typical measurement error of 1 millimeter of mercury (mmHg; SD) (Chihara, 2008). A difference as small as 1 mmHg in the mean IOP may be crucial for individuals with glaucoma, even if the vulnerability of the optic nerve is different among individuals (Chihara, 2008). It is calibrated for an average eye and measurement accuracy is therefore dependent on parameters such as corneal thickness and curvature, ocular rigidity, and tear film (Asejczyk-Widlicka and Pierścionek, 2008). There are several proposed correction algorithms for GAT measurement of IOP, but they mainly account for corneal thickness in the normal population (Chihara, 2008). Thus, patients with normal tension glaucoma may be recognized as having higher than normal IOP, and more importantly those with ocular hypertension may be recognized as being within the normal range (Copt et al., 1999).
Applanation tonometry only provides a single measurement at a point in time that may not represent an individual’s true IOP, not taking into consideration diurnal IOP variations and fluctuations that may be caused by other factors such as body posture (Malihi and Sit, 2012), blood pressure (Sung et al., 2009) or physical exercise. Mosaed et al. (2005) demonstrated that 62 percent of patients with glaucoma experienced IOP peaks outside of the office hours, when testing is normally performed. Repeated measures may be taken throughout the day; yet, the procedure is inconvenient, and not truly representative of IOP during critical time periods such as sleep, when IOP is likely at its highest (Liu et al., 2003; Kida et al., 2008). Also, non-continuous measurements may simply represent the circadian variations of IOP and not clearly depict its true nature. Regardless, several factors need to be in play when assessing an individual’s IOP when not taken continuously with regard to setting of measurement, whether at home or in the office; and the number of measurements taken and when they were obtained.

In the previous chapter, proof-of-principle was presented for the non-invasive approach to continuously measuring IOP over extended periods of time. This proof-of-principle study included a commercially available strain gauge sensor placed over the sclera in an enucleated porcine eye to measure deformation in response to pressure changes. The study demonstrated a non-corneal approach to measuring continuous IOP, aimed at providing a safer technique than a corneal approach. The device was placed on the conjunctiva over the scleral surface and could successfully correlate to true IOP. The sclera is a fibrous, elastic tissue that extends from the cornea to the optic nerve. It is the principle load-bearing tissue of the eye (Norman et al., 2010), constantly being subjected to IOP and external environmental pressure changes. Sigal et al. (2004) suggested that the impact of IOP on the sclera indirectly affects the deformation of the optic nerve head and that the biomechanical effects in the lamina cribrosa are highly influenced by scleral properties. Unlike the cornea, its stress versus strain sensitivity is much higher. In particular, IOP elevations have been shown to alter the curvature of the sclera but not that of the cornea (Pierscionek et al., 2007). Pierscionek et al. (2007) found that IOP deforms the sclera linearly providing evidence that it is the most sensitive tissue in response to IOP changes. To further the investigation into the use of the scleral surface to measure IOP changes, we tested a strain gauge sensor using live, anesthetized pigs. Thus, reporting physiologically relevant results performed on the intact eye.
The purpose of this study was designed to test the principal hypothesis that IOP measurements can be accurately recorded over time from the scleral surface of a pig eye using a strain gauge sensor, in order to assess the feasibility of continuous IOP monitoring.

3.2 Methods and Materials

3.2.1 Animals

All experiments were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care Committee by the Toronto Western Hospital, University Health Network. Nine eyes from 5-6-week old, 30-50kg Yorkshire swine were used. Prior to anesthesia animals fasted for 12 hours. Pre-medication included atropine (0.1mg/kg I.M.) and rompun (2mg/kg I.M.). Anesthesia was induced with 5 percent isoflurane in order to perform endotracheal intubation and administered with 100 percent oxygen delivered through a facemask. Eyes were anesthetized with topical 0.5 percent proparacaine hydrochloride, and corneas were kept hydrated with 0.5 percent carboxymethylcellulose sodium. Animals were kept on their side until experiments on one eye were completed and then turned over to initiate experimentation on the contralateral eye (Figure 3-1). Speculums were used to hold the eyelids open in order for placement of strain gauge sensor. Initially, the speculum was inserted and the eyes were gently washed with saline to remove and debris. Eyes were then gently wiped dry with a surgical sponge. In certain instances, two conjunctival sutures were placed approximately 2mm from superior and inferior limbal margins to control ocular alignment during IOP manipulations. Artificial ventilation was supplied with 100 percent oxygen (volume 3L min-1; rate 18 breaths min-1; Ohmeda 7000 Ventilator). Lactated ringer’s solution was administered IV. Body temperature, heart rate and oxygen saturation levels were monitored throughout the procedures. All animals were sacrificed after experimental procedures.
Animals were kept on their side until experiments on one eye were completed and then turned over to initiate experimentation on the contralateral eye.

3.2.2 Strain Gauge Sensor

The sensor (Omega Engineering, Inc., Stamford, CT) was a pre-wired strain gauge encapsulated with two wires that consists of a thin, deformable 5mm grid. The measuring grid was formed by an etching Constantan foil, a copper-nickel alloy, which was then completely sealed in a carrier medium composed of polyimide film. Two strain gauges were glued together with 100 percent cyanoacrylate as a means to minimize sensitivity to temperature by doubling the output voltage of the bridge, forming a half-bridge. The strain gauge was mounted with 100 percent ethyl-2-cyanoacrylate to the scleral, bulbar conjunctiva, approximately 1-2 mm from the limbal margin in the superior temporal region (Figure 3-2). Strain gauges were never reused and always discarded after completion of experiments. New strain gauges were selected for each eye and tested before placement on the ocular surface to verify connectivity and performance.
The eyelids of the swine will be restrained with a speculum. A pre-wired strain gauge will be positioned under the upper lid. Experiments will be performed with and without the removal of the conjunctiva. 100% ethyl cyanoacrylate is applied to one side of the pre-wired strain gauge that is then attached to the conjunctiva or the sclera. Once the strain gauge is secured, the anterior portion of the cornea is then pierced with a 27-gauge needle primed with saline and connected to a 30ml syringe on a stand with variable height. Another 27-gauge needle is inserted into the anterior chamber through the nasal limbus and connected to a pressure transducer. Adjusting the height of the syringe on the stand generates IOP changes. We have performed proof of principle experiments on one pig.

The anterior chamber was cannulated using a 27-gauge needle introduced from the temporal limbus and connected to a 30ml syringe filed with degassed water on a stand with variable height. Another 27-gauge needle was inserted into the anterior chamber through the nasal limbus primed with degassed water and connected to a pressure transducer. Adjusting the height of the syringe generated IOP changes and was recorded with a commercial software program (TracerDAQ™ Software, MicroDAQ.com Ltd., Contoocook, NH) (Figure 3-3).
Figure 3-3. Schematic Of Experimental Setup for Live Porcine Model. The pig eyes are cannulated with a 27-gauge needle inserted into the anterior chamber and connected with a silicone tube to a column filled with saline attached to a pressure transducer via a stopcock. Another 27-gauge needle attached to either a syringe pump or a reservoir of saline on a stand of variable height is also inserted into the anterior chamber and is used to control the IOP. The pre-wired strain gauges are connected into a Wheatstone bridge circuit with a combination of two active gauges (half bridge). The Wheatstone bridge configuration compensates for thermal drift. An amplifier is also connected to increase sensitivity. The strain gauges are secured with 100% ethyl 2-cyanoacrylate and the aid of the upper lid to hold the strain gauge in place during recordings. The strain gauges are not reused and the entire procedure is repeated for every eye.
IOP was increased in increments of 7mmHg from 0 to 44 mmHg (± 1.0 mmHg) and then from 44 to 0 mmHg and the surface deformation of the strain gauge sensor was recorded for each incremental step. Incremental steps were recorded for 30 seconds and a 15-minute wait period was applied for every incremental change in pressure. IOP changes were repeated three times to establish reproducibility and linearity of the strain gauge sensor. While this system is capable of continuously monitoring IOP, we analyzed data in five-minute time blocks that resulted in over 30,000 data points. This allowed us to examine whether the device was capable of distinguishing very small changes in strain arising from the dynamics of using a live model (i.e. eye blinking and body movement). A new strain gauge sensor was employed for every eye used and a baseline measurement was taken in order to determine any variation due to temperature compensation. Two strain gauges were mounted and glued to each other as a way of correcting for temperature drift. Strain gauge deformation versus change in IOP and pressure was plotted in five-minute time windows for all animals tested.

3.3 Results

Nine eyes were examined from six Yorkshire swine in this experiment. In all cases the strain gauge sensor correlated linearly with IOP variations. Figure 3-4 (A-I) shows the representative profiles of the averaged plot of IOP change with strain gauge deformation during that time session with corresponding correlation of voltage versus pressure with standard deviations. Surface deformation of the strain gauge sensor responded linearly with changes in ocular pressure in a live pig model. Data points were averaged to reduce the signal to noise ratio from 30,000 data points to 100 data points. In Figure 3-4 (B,F), blinking of the eyelid and movement of the eye, Bell’s phenomenon, as a result of anesthesia was observed, implying that the strain gauge sensor was able to record small physiological conditions. Analysis of variance gave a significant difference between strain gauge deformation and IOP manipulations (p<0.005) with linear regression coefficient ($r^2$) values ranging from 0.884 to 0.976 with increasing IOP and 0.808 and 0.983 with decreasing IOP (Table 3-1). The minimum sensitivity observed in the nine eyes tested was 0.569 mV/mmHg and the maximum was 3.16 mV/mmHg with increasing IOP. The minimum sensitivity observed was 0.450 mV/mmHg and the maximum was 3.05 mV/mmHg with decreasing IOP.
A. 

B. 

\[ y = 7.08E^{-4}x + 2.77E^{-3} \\ r^2 = 0.935 \]

\[ y = 4.50E^{-4}x + 1.44E^{-2} \\ r^2 = 0.920 \]

\[ y = 5.69E^{-5}x - 2.06E^{-3} \\ r^2 = 0.941 \]

\[ y = 7.14E^{-5}x - 6.64E^{-3} \\ r^2 = 0.965 \]
G.

H.
Figure 3-4. Recordings Of Strain Gauge Sensor in Response to IOP Changes When Applied To The Conjunctiva In A Live Porcine Model (N=9). (A-I) profiles of the averaged plot of IOP change (mmHg) with strain gauge deformation (V) during that time session with corresponding correlation of voltage versus pressure with SD. Data points were averaged over a five-minute recording period. Fifteen-minute intervals were taken between IOP increments and strain gauge recordings. Pressure was increased by 14mmHg at every incremental step. Red line represents strain gauge response; black line represents transducer.
Table 3-1. Rho Values for Increasing and Decreasing IOP Manipulations in a Live Porcine Model.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Increasing IOP (0-44mmHg)</th>
<th>Decreasing IOP (44-0mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.935</td>
<td>0.820</td>
</tr>
<tr>
<td>B</td>
<td>0.941</td>
<td>0.965</td>
</tr>
<tr>
<td>C</td>
<td>0.949</td>
<td>0.904</td>
</tr>
<tr>
<td>D</td>
<td>0.929</td>
<td>0.808</td>
</tr>
<tr>
<td>E</td>
<td>0.930</td>
<td>0.983</td>
</tr>
<tr>
<td>F</td>
<td>0.976</td>
<td>0.874</td>
</tr>
<tr>
<td>G</td>
<td>0.916</td>
<td>0.931</td>
</tr>
<tr>
<td>H</td>
<td>0.884</td>
<td>0.911</td>
</tr>
<tr>
<td>I</td>
<td>0.956</td>
<td>0.898</td>
</tr>
</tbody>
</table>
Table 3-2. Sensitivity of Strain Gauge with Increasing and Decreasing IOP in a Live Porcine Model.

<table>
<thead>
<tr>
<th>Animal ID</th>
<th>Sensitivity Increasing IOP (mV/mmHg)</th>
<th>Sensitivity Decreasing IOP (mV/mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.708</td>
<td>0.450</td>
</tr>
<tr>
<td>B</td>
<td>0.569</td>
<td>0.714</td>
</tr>
<tr>
<td>C</td>
<td>2.42</td>
<td>1.80</td>
</tr>
<tr>
<td>D</td>
<td>1.15</td>
<td>1.21</td>
</tr>
<tr>
<td>E</td>
<td>2.78</td>
<td>3.05</td>
</tr>
<tr>
<td>F</td>
<td>2.45</td>
<td>1.76</td>
</tr>
<tr>
<td>G</td>
<td>2.06</td>
<td>2.12</td>
</tr>
<tr>
<td>H</td>
<td>3.16</td>
<td>2.57</td>
</tr>
<tr>
<td>I</td>
<td>2.29</td>
<td>1.30</td>
</tr>
</tbody>
</table>

The changes in scleral curvature correlated to IOP are measured. In general, there is a good correlation between the real pressure and the results obtained from the pre-wired strain gauge. Variability in sensitivity may indicate intravariable difference in scleral thickness. The sensitivity with increasing IOP ranged from 0.569 mV/mmHg to 3.16 mV/mmHg; and with decreasing IOP ranged from 0.450 mV/mmHg to 3.05 mV/mmHg. Other factors that may affect this parameter may include setup procedures. For instance, gluing applications, whether too much or too little, may cause the strain gauge to not properly deform to direct pressure changes. It was noted that linear regression was higher with increasing pressures than with decreasing pressures. This can be explained by the biomechanical properties of the globe. The eye is inflated to pressures as
high as 50mmHg. The ability for the globe to deflate and reach equilibrium may take longer to do so, resulting in lower linear regression values as seen in Figure 3-4 (F). Figure 3-4 (E-I) show small incremental spikes during IOP recordings. Figure 3-5 shows average response of strain gauge output to IOP changes in a live porcine model. The interference was so vast causing incremental spikes throughout the recording. These spikes were noted to be in response to other electrical equipment located in the operating room. This signal-to-noise ratio may have also been caused by other factors such as variations in temperature and variations of humidity. Tearing of the eye may have caused these variations. However, it was noted that when the artificial ventilation was turned off, for a period of 10 seconds, recordings did not generate such spike like characteristics.

![Graph showing strain gauge and transducer output](image)

Figure 3-5. Electrical Interference of Ventilating Machinery on IOP Monitoring Device. Incremental spikes throughout the recording was an indication of electrical interference from other sources of machinery located in the operating room. The signal-to-noise ratio was averaged out over the 30,000 data points and although some correlation can be detected with IOP increments if is hard to justify a true response based on interference. Red line - strain gauge; black line – transducer.
Figure 3-6. illustrates the response of the scleral strain gauge to physiological parameters during recording time. Physiological parameters such as ocular movements or blinking were observed via the strain gauge response. This data was excluded based on the fact that the animal was moving frequently during recording causing constant shifting of the strain gauge. Even the use of conjunctival sutures placed two mm from the superior and inferior limbal margins did not prevent ocular movement and/or blinks. The animal had been sedated for an extensive period of time by a previous research group and anesthesia not only involved the use of isoflurane but propofol as well to maintain level of sedation. The levels of anesthesia may have been low in order to retain the animal under anesthesia for an extended period of time. The constant movement of the eye rendered a lag in response to IOP manipulations and an rho value of 0.944 for increasing IOP and 0.714 for decreasing IOP. Conjunctival sutures were employed and placed 2mm from the superior and inferior limbal margins but proved unsuccessful. The sutures were not tightened excessively to prevent additional pressure being implemented on the ocular surface.
Figure 3-6. Recording of Strain Gauge Sensor Under Repetitive Ocular Movement in A Porcine Model Under Anesthesia. Increasing IOP manipulations rendered rho values of 0.944 and decreasing IOP manipulations rendered rho values of 0.719. Repetitive ocular movements may have been a result of lack of aesthetical depth resulting in Bell's phenomenon. Conjunctival sutures were also employed to prevent movement but were unsuccessful in doing so without causing external pressure changes within the eye. Red line - strain gauge; black line – transducer.
3.4 Discussion

This project evaluates the use of a non-invasive, pressure sensor used to measure changes in intraocular pressure (IOP) in a porcine model. IOP is leading risk factor for glaucoma and is crucial in the management of this chronic ocular disease. IOP can be measured directly (manometry) or indirectly (tonometry). Applanation tonometry, using a contact method is the current clinical technique for IOP recording. However, because IOP exhibits diurnal variations it is difficult to provide reliable and repeatable measurements. It is also currently impossible to monitor IOP continuously or during sleep, even though it is acknowledged that there is great clinical value in understanding the relationship between nocturnal IOP and systemic blood pressure.

Continuous monitoring of IOP over the diurnal cycle is thought to assist with the diagnosis and management of glaucoma, ultimately preventing permanent and extensive vision loss. This study assessed the feasibility of a non-invasive, strain gauge sensor to measure changes in IOP in a live porcine model. The results presented in this study demonstrated that changes in IOP can be accurately measured by a strain gauge sensor when placed on the scleral bulbar conjunctiva. The study illustrated high linearity between strain gauge deformation and change in IOP, in spite of the strain gauge being “off the shelf” and not optimal for measurement of scleral strain. Variability between subjects for sensitivity expressed as mV/mmHg was observed and can be explained by physical parameters (i.e. scleral thickness and rigidity) of the eye within the animals used. Thus, providing a proof-of-concept for the realization of a conjunctiva/scleral mounted sensor to monitor IOP.

Elevated IOP is a risk factor for the development and progression of glaucoma (Hughes et al., 2003; Singh and Shrivastava, 2009), and lowering it remains the only proven way to arrest its advancement (Liu et al., 1998). Despite all that we know about IOP and its relationship with this optic neuropathy, there remain many unanswered questions due to the inability to properly assess IOP over long periods of time, particularly during sleep. GAT overestimates IOP for individuals with thicker corneas and underestimates it for individuals with thinner corneas. In addition, it can only be used to measure IOP while the patient is awake.

Monitoring methods of IOP are limited in their ability to continuously measure IOP over extended periods of time. Office time IOP measurements have shown to be insufficient in
determining true IOP and are limited by being unable to detect changes in evening, night and early morning hours (Holló et al., 2013). Day phasing techniques in which patients are tested every hour for a 10-hour duration are inconvenient and expensive to perform, but still give little data. Twenty-four hour phasing was reported by Moodie et al. (2010) and showed that it offered no advantage over day phasing in recognizing IOP peaks (Moodie et al., 2010). Other monitoring means include 24-hour IOP curve with hospitalization and sleep laboratory measurements, yet are both limited in requiring the patients to be woken up during measurements. All current methods of monitoring IOP are unable to obtain IOP measurements while asleep (Holló et al., 2013; Sit, 2009; Sit and Liu, 2009). More recently, Leonardi et al. (2009) developed a wireless silicone contact less sensor for continuous monitoring of IOP. The device was adapted and tested on enucleated pig eyes and demonstrated minimal invasiveness and functionality in monitoring IOP (Liu et al., 2003). The device was based on the assumption that the central corneal radius of curvature changes by approximately 3µm for every 1mmHg change in IOP in enucleated pig eyes (Liu et al., 2003). Mansouri and Shaarawy presented results of a 24-hour human trial using a wireless version of the device, the Triggerfish® (SENSIMED, Switzerland), in 13 patients with open-angle glaucoma. The authors reported the ability to monitor IOP fluctuations in patients over 24-hours with no adverse reactions other than keratitis in four patients (31 percent). Another study was also conducted employing the Triggerfish® (SENSIMED, Switzerland), reporting the safety, tolerability and reproducibility of the device in patients with glaucoma. The device showed fair to good reproducibility. Eighty percent of the patients experienced conjunctival hyperemia and 15 percent superficial punctate keratitis (Mansouri et al., 2012). Freiberg et al. (2012) analyzed the corneal thickness after overnight wear of the Triggerfish lens in 20 patients with ocular hypertension or established glaucoma. The study revealed that the device did not appear to be affected by changes in corneal thickness that normally occurs with overnight contact lens wear. The study did not assess the biomechanical properties of the cornea, including corneal hysteresis and corneal thickness. Freiberg also noted that there was a corneal inflammatory response to the wearing of the Triggerfish® contact lens sensor (Freiberg et al., 2012).

Few studies have investigated the sclera as a possible anatomical site to indirectly measure IOP. The rheological properties of the sclera are nonetheless vital because they alter in response to changes in pressure (Asejczyk-Widlicka et al., 2011) more effectively than those of the cornea. Asejczyk-Widlicka and Pierscionek (2008) demonstrated in intact porcine eyes that scleral
elasticity was 3 to 3.5 times higher than the cornea with increasing volume. These values were comparable to human eyes for a similar range of applied pressures. They also reported that there was no significant alteration in average corneal curvature when pressures were adjusted from 12 mmHg to 25 mmHg. However, there was an increased linear correlation with scleral curvature (Asejczyk-Widlicka and Pierscionek, 2008).

Limitations of the study included standardizing the amount of glue being used to bond the strain gauge to the conjunctiva. If too much glue was employed, the strain gauge would stiffen and be unable to fully bend or deform in response to the changes in IOP. Too little glue resulted in instability of the strain gauge. As a result, excess glue on the strain gauge led to differences when comparing increasing and decreasing IOP values. Although all values showed high correlation between the strain gauge deformation and IOP manipulations, with rho values greater than 0.800, differences were noted in four of the nine eyes, in which the final strain values following decreasing IOP did not return fully to baseline.

The cornea provides the major refractive power for the eye and needs to maintain its shape in order to minimize vision disturbances. The greater flexibility of the sclera in comparison to the cornea, allows it to change more readily in response to strain such as those induced by changes in pressure. This proof-of-principle study demonstrated that a scleral mounted strain gauge was capable of continuous IOP monitoring and that changes in IOP were accurately assessed by measuring changes in the radius of curvature of the sclera in a live porcine model.
Chapter 4  Comparison of the Cellular Response to Overnight Contact Lens Wear on the Sclera and Cornea as an Initial Characterization for the use of an IOP Monitoring Device
4 Summary

The purpose of the present study is to investigate the overnight inflammatory response to a silicon hydrogel contact lens mounted on the superior, scleral bulbar conjunctiva, and compare it to overnight lens wear on the cornea. The contact lens was used as a surrogate for the carrier material for a 24-hour IOP monitoring device. Eight participants were recruited to a sleep lab for 3 non-consecutive visits. The 1st visit consisted of overnight sleep (8 hrs) with no contact lens in place (baseline); the second visit consisted of overnight sleep with a hydrogel contact lens fitted bilaterally to the cornea; and the third visit consisted of overnight sleep with a hydrogel contact lens placed bilaterally on the upper bulbar conjunctiva, beneath the upper lid. Immediately upon awakening, participants had the lenses removed and their eyes washed using a non-contact irrigation system. The expression of the C3a receptor (C3aR), CD95 (Fas, cell death inducing receptor), CD54 (ICAM-1), CD66b (degranulation marker), and CD45 (Pan leukocyte marker) on polymorphonuclear leukocytes (PMN) was evaluated by flow cytometry for each visit and is reported as arbitrary fluorescent units (AFU). Flow cytometric analysis of PMN revealed a significant decrease in the expression of C3aR after overnight contact lens wear on the cornea (10 ± 7 AFU, p < 0.01) as compared to overnight lens wear over the sclera (33 ± 19 AFU) and baseline (33 ± 11 AFU). For all visits CD95, CD54, CD45 and CD66b remained at background levels with no significant difference in expression. These results suggest that overnight lens wear on the cornea alters the expression of C3aR, a receptor important in the regulation of the leukocyte inflammatory response, indicating some dysregulation of the complement system. PMN response to overnight contact lens wear on the superior, bulbar conjunctiva was similar to the control, no contact lens condition. Silicone hydrogel contact lenses, approved for overnight wear on the cornea, may be safely mounted on the upper bulbar conjunctiva and presents a promising carrier material for a 24-hour IOP monitoring device.
4.1 Introduction

The dynamics of IOP in relation to overall changes to the diurnal cycle are still not fully understood. Comparatively, little is known regarding the circadian nature of IOP fluctuations and variations. Changes in IOP may be affected by physical activity, alterations in cerebral vasculature and pharmacotherapy, but the extent of their influence still need to be assessed in order to understand meaningful trends in IOP (Twa et al., 2010). Current practice for obtaining diurnal IOP measurements involve overnight stays in hospitals or sleep laboratories. Not only do patients need to be awoken from a sleep, supine position to an alert, sitting position; but IOP is also acquired by applanation of the cornea (Goldmann), which is highly dependent on corneal parameters (Nosch et al., 2010; Hong et al., 2013; Fogagnolo et al., 2006) such as thickness, curvature, structure and axial length. This reduces the reliability and repeatability of the real IOP profile of a patient. Recently, Leonardi et al. developed a wireless strain gauge sensor embedded in a silicon contact lens that measures deformation of the cornea associated with IOP changes (Leonardi et al., 2004; Leonardi et al., 2009). The device was based on the assumption that central corneal radius of curvature changes by approximately 3µm for every 1mmHg change in IOP in enucleated pig eyes. Mansouri et al. (2012) presented results of a 24-hour human trial using a wireless version of the device, the Triggerfish® (SENSIMED, Switzerland), in 21 patients suspected of having glaucoma and in 19 patients with established glaucoma. The authors reported the ability to monitor IOP fluctuations in patients over 24-hours with fair to good reproducibility, yet with 80 percent of their patients developing conjunctival hyperemia and 15 percent keratitis. Large clinical trials remain to be performed and the relationship between strain and IOP still needs to be established.

In the previous chapters, we have characterized a strain gauge sensor that can accurately measure IOP from the scleral and conjunctival surface in enucleated porcine eyes and in a live pig model that may be used for extended periods of time. Unlike the cornea, the sclera is the principle load-bearing tissue of the eye (Normal et al. 2010) and is constantly being subjected to IOP and external environmental pressure changes. The sclera’s elasticity and its stress versus strain capability is much higher than that of the cornea (Pierscionek et al., 2007; Asejczyk-Widlicka et al., 2011). In particular, IOP elevations may alter the curvature of the sclera but not that of the cornea (Pierscionek et al., 2007). A study conducted by Pierscionek et al. (2007) found that IOP
deforms the sclera linearly providing evidence that it is the most sensitive tissue in response to IOP changes. To further establish the scleral strain gauge sensor as a monitoring device in clinical practice it would have to be embedded to a carrier material, such as a contact lens, that could be worn on the conjunctiva overlying the sclera. The purpose of the present study is to investigate the overnight inflammatory response to a silicon hydrogel contact lens mounted on the superior, scleral bulbar conjunctiva, and compare it to overnight lens wear on the cornea. The contact lens was used as a surrogate for the carrier material for a 24-hour IOP monitoring device.

Since glaucoma progression decreases by 10 percent for every mmHg decrease in IOP, it has been proposed that obtaining 24-hour IOP measurements may alter the risk profile of a patient for disease progression (Weizer et al., 2006). Hence, the development of a continuous, non-invasive IOP monitoring device, particularly during sleep and regular activities in humans, that provides accurate IOP profiles independent of corneal properties would be fundamental in understanding and identifying risk factors for glaucoma and its progression. We hypothesize that extended contact lens wear would be safer, causing a lesser inflammatory response, when worn over the sclera, and that a strain gauge sensor measuring IOP changes over the scleral tissue would provide a more accurate and sensitive result than when placed on the cornea. These proof-of-principle experiments are an essential step to the realization of a safe, scleral mounted, 24-hour IOP recording device.
4.2 Materials and Methods

There is currently very little known about cells in the closed-eye environment. Neutrophils are known to invade the eye (or extravasate) during sleep and may react to the presence of a biomaterial worn during sleep. This contact may induce an inflammatory response that has deleterious effects on the material’s biocompatibility. To assess the potential for inflammation in the anterior eye, the following protocol is suggested.

Upon awakening, cells are collected in phosphate buffer saline (without Ca$^{2+}$ and Mg$^{2+}$) using the Ocular Surface Cell Collection Apparatus (for optimal cell collection, eyes need to remain shut until immediately prior to cell collection). Cells are collected in 10 mL PBS (each eye takes about 30 sec). 5mL of serum containing medium is then added to collected cells (final concentration of FBS is 10%) and cells are brought to the lab for immunostaining. Cells are centrifuged and resuspended in 10% FBS-Medium. Collected cells are then incubated with fluorescently labeled antibodies:

- combination 1: FITC-CD11b, PE-CD54 and PE-Cyt 5-CD45
- combination 2: FITC-CD66b, PE-CD33 and PE-Cyt 5 CD45

for 20 minutes in the dark at room temperature. Negative controls (IgG isotypes) are also run for each experiment. Following incubation, samples are then diluted in Hepes Tyrode Buffer and fixed in paraformaldehyde (1% final concentration). Samples are stored in the fridge and analyzed within 5 days on the flow cytometry.

The level of fluorescent intensities will be compared between baseline and nights when contact lenses were worn on the cornea and conjunctiva. If enough cells are collected, to provide additional information on the state of leukocytes, it may be advisable to also have a stimulated sample (positive control) for both eyes: stimulus such as phoebe ester microstate (PMA), flip or LPS may be used during incubation with antibodies. Cells have been to become refractory following material contact and thus it may be important to verify their potential for activation.

Choice of markers known to be unregulated upon inflammatory stimulus:

CD54: ICAM-1, adhesion molecule
CD66b (previously referred to CD67): Europhile degranulation marker

CD45: pan leukocyte marker: used to identify leukocyte population

This study prospectively investigated the expression of C3a receptor (C3aR), CD95 (Fas, cell death inducing receptor), CD54 (ICAM-1), CD66b (degranulation marker), and CD45 (Pan leukocyte marker) on polymorphonuclear leukocytes (PMN) from the ocular surface of healthy individuals after overnight contact lens wear on the conjunctiva and cornea. All participants were recruited from the Centre for Contact Lens Research at the School of Optometry, University of Waterloo, and Waterloo, Canada. Informed consent was obtained from each participant after explanation of the nature and possible consequences of the study. All participants were treated in accordance with the tenets of the Declaration of Helsinki. Ethics clearance was obtained through the Office of Research Ethics at the University of Waterloo and the Office of Research Ethics at the University of Toronto.

4.2.1 Participants and Study Visits

Eight participants (5 females and 3 males; mean age 35) were recruited to a sleep lab for 3 non-consecutive visits. Previous to enrollment, participants were screened to determine eligibility based on ocular history, slit lamp biomicroscopy, previous contact lens wear experience, and trial lens fitting. The first visit consisted of overnight sleep (8 hrs) with no contact lens in place (baseline); the second visit consisted of overnight sleep with a silicone hydrogel contact lens (76 percent Iotafilon A, 24 percent water) fitted to each cornea; and the third visit consisted of overnight sleep with the same type of silicone hydrogel contact lens placed bilaterally on the upper bulbar conjunctiva, beneath the upper lid. Each visit was scheduled for participants to arrive to the sleep lab at 10 pm with lenses fitted at 11pm for sleep until 7 am the next morning, an eight-hour duration. Visits were separated by at least 72 hours, during which no lenses were worn by the participants.
Table 4-1. Study Procedures and Duration for Overnight Contact Lens Wear on the Bulbar Conjunctiva and the Cornea. A total of 4 visits to the CCLR were required by participants. The first visit is a screening visit and is not an overnight visit that lasts 60 minutes. The second visit is a baseline visit and requires no use of contact lenses but an overnight sleep at the CCLR. The third and fourth entails contact lens fitting to participants.

<table>
<thead>
<tr>
<th>Study visit name</th>
<th>Study procedures</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening visit</td>
<td>Eligibility confirmed, Air Optix™ Night &amp; Day™ lenses trial fitted Note: These soft contact lenses are readily available and marketed in Canada</td>
<td>1 hour</td>
</tr>
<tr>
<td>Fit-refining visit</td>
<td>Only scheduled if additional lenses need to be ordered to confirm fit</td>
<td>1 hour</td>
</tr>
<tr>
<td>Visit 1 (Baseline)</td>
<td>Overnight sleep with NO contact lenses placed in eyes Eyes immediately washed with the Ocular Surface Cell Collection Apparatus (OSCCA) upon awakening</td>
<td>8 hours</td>
</tr>
<tr>
<td>Visit 2 (24 hours later)</td>
<td>Overnight sleep with contact lenses placed on cornea Eyes immediately washed with the Ocular Surface Cell Collection Apparatus (OSCCA) upon awakening</td>
<td>8 hours</td>
</tr>
<tr>
<td>Visit 3 (72 hours later)</td>
<td>Overnight sleep with contact lenses placed on sclera Eyes immediately washed with the Ocular Surface Cell Collection Apparatus (OSCCA) upon awakening Measurements taken with study Exit from study</td>
<td>8 hours</td>
</tr>
</tbody>
</table>

4.2.2 Cell Processing

Immediately upon awakening, participants had the lenses removed and their eyes washed using the Ocular Surface Cell Collection Apparatus (OSCCA) a non-contact irrigation system described previously (Peterson et al., 2011) (Figure 4-1). The OSCCA was developed at the Center for Contact Lens Research, School of Optometry, University of Waterloo, Waterloo, Canada. In short, the OSCCA irrigates the anterior eye with sterile saline, washing loosely adherent cells from the ocular surface and collecting them in a sterile test-tube. Once the contact lens was removed from the eye, it was gently irrigated with PBS that was then transferred to the same test-tube with the irrigation wash solution from the participant. The combination of the wash from the anterior eye and that from the contact lens of each participant was then
centrifuged at 1200 rpm for seven minutes at 21° Celsius. The solution was then aspirated and resuspended in DMEM with 10 percent fetal bovine serum (FBS). The cells collected from the left and the right eye of each participant were pooled together. An equal volume of cell suspension and trypan blue was used to determine cell counts of PMNs and epithelial cells via a hematocytometer for each participant after each visit. All cytokines were measured in a blinded fashion (e.g., clinical data were not known when the measurements were performed). The expression of the C3a receptor (C3aR), CD95 (Fas, cell death inducing receptor), CD54 (ICAM-1), CD66b (degranulation marker), and CD45 (Pan leukocyte marker) on polymorphonuclear leukocytes (PMN) was evaluated by FACScan flow cytometer (Becton Dickinson) for each visit and was reported as mean arbitrary fluorescent units (AFU). PMNs were distinguished from other leukocytes by their characteristic side scatter. Results were displayed on a side-scatter histogram.

**Figure 4-1. Diagram of Cell Collection with the Ocular Surface Cell Collection Apparatus (OSCCA)** The OSCCA apparatus is formed by a funnel 5 cm in diameter through which a 21 G blunt needle is secured. The needle passes perpendicularly via the funnel near the base, allowing for the insertion of a collection tubule. Irrigation solution is pumped through the needle by a peristaltic pump. A test tube is securely screwed to the funnel allowing for collection of cell suspension from ocular surface. (Peterson et al., 2010).
4.2.3  Contact Lens Material

The contact lens material used in the study was an FDA approved silicone hydrogel extended wear spherical lens that was approved by Health Canada for extended wear and was readily available in Canada. The contact lens was an Air Optix Night & Day Aqua™, manufactured by CIBA Vision. It is a lotrafilcon material with 24 percent water content that has been approved to be worn for up to 30 nights of continuous wear. The contact lens was chosen because of its large diameter of 13.8 mm that would more likely comfortably fit underneath the upper lid in the bulbar conjunctiva for overnight wear.

4.2.4  Statistical Analysis

All results are reported as mean AFUs ± standard deviation (SD). To evaluate the significance of the differences in cytokine activation across all visits an analysis of variance (ANOVA) was performed followed by a multiple pair-wise comparison using the Tukey test for continuous variables with Prism Version 5.0a (Graph Pad, LaJolla, CA). Overnight contact lens wear on the cornea and overnight contact lens wear underneath the lid on the bulbar conjunctiva was compared with no overnight contact lens wear. Significant difference between visits was also reported. A significant level of p value of ≤ 0.05 was used for all analysis.
4.3 Results
Eight participants with a mean age of 35 (ranging from 22 to 53 years of age) participated in the study. None of the participants experienced any ocular adverse reactions during the course of the study. Participants reported no discomfort overnight when having the contact lens placed either on their corneas or bulbar conjunctiva. One participant (ID 001) had to repeat a baseline, no overnight contact lens wear, due to an insufficient number of PMNs. Another participant (ID 008) had insufficient cell numbers when the contact lens was placed on the conjunctiva but was unable to repeat the visit and therefore their data was not included in the statistical analysis. Cells were counted manually via a hemocytometer as soon as possible after collection, to minimize any potential damage or distortion. C3aR, a receptor for the chemotactic and inflammatory peptide anaphylatoxin C3a, showed a significant decrease in expression for contact lens wear on the cornea (AFU ± Stdev) (11.6 ± 4.5) in comparison to contact lens wear on the conjunctiva (42.4 ± 23.2) and baseline (34.9 ± 12.2). CD95, a cell death receptor protein that regulates tissue homeostasis by inducing cell death, showed no significant difference between baseline, contact lens wear on the cornea and contact lens wear on the conjunctiva. CD66, a protein involved in cell adhesion and growth, neutrophil activation and signaling, showed no significant difference between baseline, contact lens wear on the cornea and contact lens wear on the conjunctiva. CD54, an intercellular adhesion molecule, showed no significant difference between the three states nor did CD45, a leukocyte common antigen. Cell counts were reported as the total number of live cells stained with Trypan Blue (0.4 percent) (Invitrogen) (Table 4-2 and Figure 4-2).
There was a distinct increase in PMN cell count when comparing baseline and overnight contact lens wear on the cornea and conjunctiva (Figure 4-3). Percentage of tear film neutrophils in the cell collection for baseline, overnight contact lens wear on the cornea (p < 0.01) and overnight contact lens wear on the conjunctiva (p < 0.01) in comparison to the number of monocytes and lymphocytes after an 8-hour sleep period. PMN activation was apparent under closed eye conditions during sleep. Contact lenses worn on the cornea and conjunctiva overnight showed increase expression of PMN in compared to baseline. A higher number of characterized PMN were found on the cornea after overnight contact lens wear than the conjunctiva, but was not significant. Monocyte activation across the study was not apparent as well as lymphocyte activation. PMN aggregation to the cornea and conjunctiva could be explained as an inflammatory response indicative of a foreign object being placed on the ocular surface.
<table>
<thead>
<tr>
<th>PARTICIPANT ID</th>
<th>CONTACT LENS (CL) ORIENTATION</th>
<th>NO. PMNs $\times 10^9$/CELLS/ML</th>
<th>NO. EPITHELIAL CELLS $\times 10^9$/CELLS/ML</th>
</tr>
</thead>
<tbody>
<tr>
<td>001</td>
<td>No CL</td>
<td>178</td>
<td>6</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>CONJUNCTIVA</td>
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<td>5</td>
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<td></td>
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<td>2</td>
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<tr>
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</tr>
<tr>
<td></td>
<td>CORNEA</td>
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<td></td>
<td>CONJUNCTIVA</td>
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</tr>
</tbody>
</table>
A.

C3aR

B.

CD95

C.

CD66
Figure 4-2. Illustration of the AFUs (± Standard Deviation) of PMNs of various cell surface markers. Flow cytometric analysis of PMN revealed a significant decrease in the expression of (A) C3aR after overnight contact lens wear on the cornea (10 ± 7 AFUs) as compared to overnight contact lens wear on the conjunctiva (33 ± 19 AFUs, p < 0.01*) and the baseline (33 ± 11 AFUs, p < 0.01**) response. For all visits, (B) CD95 remained at background levels and there was no significant difference in (C) CD66b, (D) CD54, and (E) CD45 expression.
Figure 4-3. Percentage Of Tear Film Neutrophils In The Cell Collection For Baseline, overnight contact lens wear on the cornea (p < 0.01*) and overnight contact lens wear on the conjunctiva (p < 0.01**) in comparison to the number of monocytes and lymphocytes after an 8-hour sleep period. PMN activation was apparent under closed eye conditions during sleep. Contact lenses worn on the cornea and conjunctiva overnight showed increase expression of PMN in compared to baseline. A higher number of characterized PMN were found on the cornea after overnight contact lens wear than the conjunctiva, but was not significant. Monocyte activation across the study was not apparent as well as lymphocyte activation. PMN aggregation to the cornea and conjunctiva could be explained as an inflammatory response indicative of a foreign object being placed on the ocular surface.
4.4 Discussion

We have demonstrated that overnight contact lens wear alters not only the number of PMNs on the ocular surface but also the concentration of inflammatory mediators in the tear film. Our main objective was to examine the cellular changes to the sclera/conjunctiva during overnight contact lens wear, and compare the response to both overnight wear of the contact lens on the cornea, and no contact lens wear.

Contact lenses can be worn on a daily or extended wear basis. In this study the lenses were worn on both a daily and extended wear basis (overnight). Adverse events and/or complications in daily wear of soft contact lenses are extremely rare. When contact lenses are worn on a daily wear basis there is a small risk of an adverse event compared to not wearing contact lenses. When contact lenses are worn on an extended wear basis, there is a significantly increased risk of an adverse reaction compared with wearing contact lenses on a daily wear basis.

There is no literature on the reaction of the sclera to contact lens wear, other than anecdotal case reports of missing lenses being found under the upper lid, and therefore on the sclera. However it is noted that there is little known response in such cases and from clinical experience. It is anticipated that there will be a lesser response to contact lenses on the sclera than on the cornea. The cornea is one of the most highly sensitive tissues of the eye. The sclera, which is covered by a soft, clear tissue, the conjunctiva, may be less sensitive than the cornea. Therefore, contact lenses that are placed on the sclera may cause less sensation than contact lenses placed upon the cornea.

In this study, we report a decrease in the complement anaphylatoxin C3aR in overnight contact lens wear on the cornea in comparison to no lens wear and overnight contact lens wear on the conjunctiva. C3 and its split product, C3a, are present in low levels in the cornea. The effector generated by the complement activation is the anaphylatoxin C3a that is elicited via its receptor that is associated with innate immunity and inflammation. The observed down regulation of C3aR in overnight contact lens wear on the cornea may indirectly indicate that inflammatory and/or complement activation was present. One of the main roles of the complement system is to remove the waste of inflammatory injury (Lim & Lappas, 2012). A down regulation of C3aR may further indicate an inability of the corneal surface to properly remove the excess of activated
complement, where immune complexes gather, prompting an inflammatory response (Lim & Lappas, 2012). Whereas no contact lens wear and contact lens wear on the conjunctival tissue did not demonstrate this incapability. We did not evaluate C3a levels in the study and no previous study has demonstrated the expression of C3aR in the ocular surface after overnight contact lens, so it is difficult to conclude as to whether the decreased expression of overnight corneal contact lens wear is an inflammatory response or a protective one. C3aR has been implicated as a novel complement in mediated signaling in retinal degeneration (Yu et al., 2012) and in excessive inflammatory disease such as preeclampsia (Lim & Lappas, 2012). It has been documented by Kida et al. that corneal parameters do change during the nocturnal period causing swelling of the cornea at night due to a higher hydration state (Kida et al., 2006).

While no other studies have described C3aR in overnight contact lens wear, there are data on PMNs in contact lens use. Thakur and Wilcox showed that during one night of sleep with contact lenses that the number of PMNs significantly changed when compared to no lens wear (Thakur and Willcox, 2000). Both overnight contact lens wear on the cornea and conjunctiva demonstrated a significant increase in PMN numbers in comparison to no overnight contact lens wear. Again, it would seem that the recruitment of PMNs to the ocular surface serves as an effective defense against pathogens in the closed eye environment (Thakur and Willcox, 2000; Tan et al., 1993).

In conclusion, our study aimed to determine whether overnight contact lens wear induced variable differences at the cellular level when placed upon the cornea compared to the conjunctiva. There was a distinct difference in regard to complement activation between having the contact lens placed on the cornea versus the conjunctiva in a closed eye environment. This significant difference may indicate that wearing a contact lens on the bulbar conjunctiva during sleep may cause less of an inflammatory response than having one placed on the cornea, but further studies are required to fully elucidate the underlying cause. Collectively, these data demonstrated the difference between corneal and conjunctival reactions for overnight contact lens wear and ultimately will have particular relevance in establishing a 24-hour non-invasive non-corneal contact lens IOP monitoring device.
Chapter 5  Concluding Summary: General Discussion and Future Direction - A Paradigm Change in Glaucoma Care
5 Concluding Summary

Glaucoma is an optic neuropathy often associated with increased IOP that can lead to impaired vision. Symptoms include gradual loss of vision and in extreme cases blindness. It has been estimated that by 2020, glaucoma will affect more than 50 million people worldwide and have caused bilateral blindness in 11.2 million people in North America (Quigley and Broman, 2006). The primary goals of glaucoma management are to avoid nerve damage and maintain a low IOP in order to preserve vision and attain a patient’s quality of life. IOP is a major risk factor for glaucoma, however, we know that throughout a 24-hour period it is not constant and fluctuates throughout the day and night. This results in the clinical dilemma that, changes in IOP, particularly during sleep, in patients with glaucoma are not accurately evaluated during the regular practitioners’ office hours when diagnosis and management are provided. Stabilization of IOP provides the only therapeutic path of halting disease progression.

IOP is the balance between the aqueous humor production and outflow. Aqueous humor circulates throughout the anterior chamber, exiting via the trabecular meshwork into the Schlemm’s canal and the episcleral veins. An increase in trabecular resistance outflow or an increase in episcleral venous pressure causes an increase in IOP. An increase in IOP along with visual field abnormalities and optic nerve neuropathy, is the hallmark of glaucomatous optic neuropathy. Yet, there are certain individuals that experience an elevation in IOP but show no signs of visual abnormalities or optic nerve cupping. Also, individuals with neuropathy may experience no signs of high IOP. Thus, elevated IOP is not the only diagnostic factor. Many individuals with IOP above the “clinically” normal limit, experience normal vision. Therefore, the development of a human continuous IOP monitoring device during day and night would be beneficial. To date, there remains no commercially available non-invasive human device that can reliably and accurately measure IOP throughout the 24-hour period. There is an experimental contact lens device available in Europe, but there are significant disadvantages using a silicon contact lens on the cornea, one of them being corneal swelling, adherence and/or inflammation.

IOP is measured clinically by depressing the cornea. Yet, we have presented an approach to monitoring IOP, allowing for measurements to be taken throughout a 24-hour period with minimal invasiveness by using a strain gauge sensor attached to the sclera. The sclera is a
fibrous, elastic tissue that extends from the cornea to the optic nerve. It is the principle load-bearing tissue of the eye, constantly being subjected to IOP and external environmental pressure changes. Unlike the cornea, its stress versus strain capability in terms of elasticity is much higher. In particular, IOP elevations may significantly alter the curvature of the sclera but not that of the cornea. A study conducted by Pierscionek et al. (2007) found that IOP deforms the sclera linearly providing evidence that it is the most sensitive tissue in response to IOP changes. Having established proof-of-principle that a scleral mounted strain gauge was able to accurately measure IOP changes in a live pig model, the next stage was to prove that it is safe to use a hydrogel material, to encase the strain gauge, for extended periods of time on the human scleral conjunctiva. Contact lenses are the most widely accepted noninvasive carrier vehicle for safe ocular use. The main principle property sought in contact lens materials in addition to required optical properties and chemical stability is oxygen transmissibility that meets the metabolic requirements of the cornea. For oxygen permeability, contact lens materials are often made of silicone components. Yet, pure silicone contact lenses are prone to surface problems and discomfort because they have a strong tendency to adhere to the cornea (Montecelli et al., 2005). Hydrogel contact lenses are not prone to adhering to the cornea because there is little tear exchange under the lens allowing most of the oxygen that reaches the cornea to permeate through the lens. Because we have demonstrated that a strain gauge can measure IOP via surface deformation that is equivalent to true IOP when placed on the sclera, the goal is to embed a strain gauge in a hydrogel material that can be worn on the conjunctiva overlying the sclera. Yet, we need to further our understanding of the impact that hydrogel materials would have on the sclera and examine what is happening to the epithelium at a cellular level. The epithelial layer of the eye is responsible for maintaining an external barrier against ocular damage and infection. Examination of this tissue is crucial for safety when contact lens wear is used. Since IOP during sleep is an important part of knowing an individual’s true IOP, it is beneficial to examine what these responses would be. Extended contact lens wear would be safer, causing a lesser inflammatory response, when worn over the sclera, and that a strain gauge sensor measuring IOP changes over the scleral tissue would provide a more accurate and sensitive result than when placed on the cornea.

Currently, there is insufficient information on nocturnal/sleep supine IOP recordings as well as the lack of continuous IOP monitoring devices. This problem has been recognized for more than
half a century and attempts to overcome this limitation have been proposed. Several attempts have been made to establish a medical device that can measure IOP over an extended period of time without jeopardizing patient comfort and ocular health. Yet, they have all resulted in failure either because of design flaws, difficulty with noise or inability to properly measure IOP leading to artifactual measurements. In addition, at the time that most of these devices were designed, such as those of Green and Gilman (1979) and Cooper (1974), the technology was not advanced in terms of biomaterials, fabrication and structure design. Most recently, Leonardi et al. (2009) attempted to reintroduce the concept of measuring continuous IOP using a strain gauge embedded in a contact lens. The authors employed a silicone-based contact lens to measure corneal deformation with IOP changes, indicating a 3µm change in radius of curvature for every 1mmHg in IOP. The design overlooked physiological parameters, perturbations caused by movements of the eye and blinking of the eyelid. Movement of the contact lens sensor on the cornea, and how this might affect the recording signal, was never documented or discussed. Moreover, the silicone contact lenses would ultimately adhere to the patient’s cornea with overnight or extended 24-hour wear, potentially causing corneal swelling, as confirmed by reports from Mansouri et al. 2012. In addition, the contact lens sensor only comes in three different corneal sizes (small, medium, large), the precision and measurement may be affected by the intra-variability of corneal thickness, corneal diameter, corneal radius, curvature and corneal rigidity. On the basis of previous IOP monitoring devices, the requirements for a clinically suitable IOP monitoring device would include factors such as non-invasiveness, micro-electrical mechanical systems (MEMS) technology that would enable remote accessing and biocompatibility that would render it safe for extended wear. What is needed to confront the issue of glaucoma management is a device that can be placed on the eye, non-invasively, that will record IOP in any given patient over various time frames and activities. A current example of a similar medical device would be a cardiac Holter monitor that is able to record the electrical activity of the heart and heart rate over a 24-hour period.

This thesis aimed to provide the proof-of-concept that a strain gauge sensor placed on the sclera can reliably correlate to true IOP and its changes, for an extended period of time. The method is based on indirect continuous monitoring of IOP due to deformation of the scleral curvature. The sclera has been reported to exhibit greater changes in response to pressure changes than the cornea. The results presented in this thesis show that a pre-wired strain gauge is sufficiently
sensitive to measure IOP manipulations in enucleated porcine eyes (n=14) and in a live porcine model (n=9). The ability to correlate linear IOP changes and scleral deformation, as measured by a strain gauge, proves its ability to monitor IOP. To decrease the invasive nature of an IOP monitoring device to be placed on the eye, a carrier material such as a contact lens would be required. A contact lens would be the ideal vehicle for continuous IOP monitoring because insertion and removal are easy. The last section of this thesis examined the inflammatory response of wearing a pre-approved hydrogel-silicone contact lens on the sclera, bulbar conjunctiva. Because funding as a PhD project is limited, and the ability to take this work to an industrial level of having a strain gauge manufactured and embedded into a contact lens was restrictive, the only plausible solution was to examine the inflammatory response of wearing the proposed carrier material, hydrogel-silicone contact lens, on the sclera, bulbar conjunctiva. By examining the cellular response to wearing a pre-approved contact lens on the conjunctiva overnight, the research provided further proof that a scleral/conjunctival monitoring device embedded in a contact lens material would be plausible and biocompatible from a medical device perspective. As a negative control, the inflammatory response of wearing the same contact lens on the cornea overnight was compared. The results were surprising in demonstrating a down regulation of C3aR on overnight corneal contact lens wear. Overnight contact lens wear on the conjunctiva showed no change from baseline, that is no overnight contact lens wear. This indicated that the overnight cornea contact lens wear may promote the induction of the complement cascade, a proinflammatory response. Again, this was a proof-of-concept that having a strain gauge positioned for IOP monitoring from the sclera may prove less harmful from an inflammatory perspective than a corneal contact lens sensor. Not to mention that a sensor placed on the conjunctiva, underneath the lid may be more comfortable for the patient as well as unobstructive to vision as opposed to a sensor place on the cornea.

Although the research of this PhD thesis was carefully prepared, reaching its aims, I am still aware of its limitations and shortcomings. First, there are several disadvantages to not having access to a ready-made sensor that was designed and fabricated within a contact lens type material. These trials were proof-of-concept, providing a potential design for a scleral-based IOP monitoring device. The development of a prototype would grant regulatory approval for proof-of-clinical utility. A prototype would allow for proper examination of issues that may affect recording signal such as thermal compensation when placed on the eye; physiological parameters
such as blood pressure, ocular pulse, temperature under the lid; and perturbations caused by blinking. Moreover, a prototype would allow for a 24-hour examination. Although the pre-wired strain gauge was tested on a live porcine model for extended periods of time, 24-hour measurements were not completed because of the inability to do so with the current design. A wireless strain gauge fabricated onto a contact lens material would have been optimal. Further experiments including animal glaucoma models would have helped to further validate the design/concept. In addition, there were limitations to having the strain gauge permanently glued to the eye. The method was invasive and strain gauges could not be recycled or used repeatedly in the same eye for a long period of time. Gluing of the strain gauge was difficult because too much glue caused inability of the strain gauge to deform while too little glue did not allow for stable mounting to the tissue. Without question, future directions would focus on sensor design and fabrication. Microfabrication of polymer substrates such as polyethylene terephthalate polymer, which is commonly used in contact lens, would have to be thoroughly investigated due to the material’s thermal and mechanical limitations. Designing a wireless component would also be beneficial and more comfortable for the patient than a wired approach.

The need for a continuous, non-invasive IOP monitoring device is obvious. Recording IOP throughout the 24-hour period has been the subject of research for decades. Although several functioning and practical sensors have been designed the have not been approved for use in North America and thus the need continues. It is likely that such a device, if properly executed, would change the way we manage a disease that affects millions worldwide. Not only would this grant us more information on IOP and its circadian nature, but it would also target the field of personalized medicine. By monitoring IOP, clinicians would be able to monitor the outcome of therapeutic interventions and surgical procedures on IOP. Of course, regulatory approval and widespread adoption of this technology will require further experiments to establish pre-clinical evaluations of the IOP measuring device as well as the efficacy and biosafety of selected carrier materials and ocular bio-adhesive formulations that will adhere the pressure sensor to the sclera. If successful, the results of this study will help to further enable prototype development and human clinical trials. These proof-of-principle experiments have illustrated the ability of a non-optimized, commercially available strain gauge to act as a sensor to IOP changes. Furthermore, studies point to the fact that the sclera can be used to adequately measure pressure changes in the eye and therefore could convincingly be used for the placement of a continuous IOP monitoring
device. This is an essential step to the realization of a safe, scleral mounted, 24-hour IOP recording device that will significantly impact the way glaucoma is diagnosed and managed.

5.1 Future Direction

IOP is a major risk factor for glaucoma and knowledge of the diurnal, including nocturnal, IOP curve of a patient who suffers from this neurodegenerative disease will provide useful information for future management. Although the development of a 24-hour IOP monitoring device does not have the capability of “curing” glaucoma, it does have the potential to transform the way we manage this debilitating disease. Millions of individuals are affected by vision loss as a result of glaucoma. Early diagnosis is essential in providing proper therapeutic care, either medically or surgically, to prevent ongoing progression. The challenge is in diagnosing the disease early. IOP is easily measured in the clinical setting. Yet, the measurements obtained during clinician office hours may not be representative of the true IOP profile of an individual. Continuous IOP measurements during the diurnal/wake and nocturnal/sleep time are needed in order to establish an individual’s IOP profile. By obtaining a series of IOP measurements during the 24-hour period at various body positions, supine and sitting, and during various activities, will give an insight or portray the true nature of a patient’s susceptibility to developing or advancing glaucoma.

This dissertation provided proof-of-principle that a pre-wired, commercial strain gauge deforms linearly to changes in IOP. The framework of this thesis provides a stepping-stone to future fabrications of a scleral strain gauge sensor. Initially the question was whether a strain gauge could measure changes in the sclera in response to IOP manipulations. Chapter 2 and Chapter 3 demonstrated in enucleated porcine eyes and in a live pig model that the change in scleral curvature correlated between the real pressure and the results obtained from the sensor. Future directions would involve the fabrication of a strain gauge sensor embedded into a silicone-hydrogel contact lens. Yet, the complexity of the latter requires the careful consideration of various factors that include: (1) knowing of the major variables involved in outflow facility; (2) design specifications; (3) electronics and wireless communications; (4) stability; (5) ergonomics; and (6) cost.
The success of a future scleral strain gauge sensor would require the understanding of the variables involved in outflow facility in glaucoma. As previously stated, several factors play a role in the maintenance of IOP. IOP is balanced by the formation and output of aqueous humor. Outflow facility and rigidity of the eye all play vital roles in this balance. A strain gauge would be required to monitor IOP from very low ranges to high ranges. It would be of beneficial use if the monitor could measure other variables that affect IOP or that can be recorded. Systemic and ocular blood pressure would be of value in recording and comparing to a given IOP measurement at a given time of day. The ability to record low and high values of IOP would provide information into fluctuations and variations.

The wide diversity and sophistication of biomaterials currently used in medicine illustrates the significant advances in technology that have occurred in the past decade. For more than 50 years, scientists have been trying to develop a non-invasive IOP monitoring device that could record continuously for extended periods of time. These devices were prone to inadequacy because of design flaws. Common problems experienced with the previous IOP monitoring devices included difficulty with signal-to-noise ratio, a measure of signal strength in relative to background noise. The recordings in a live pig model also demonstrated a minimal amount of electrical interference. This was expected especially since an off-the-shelf strain gauge was being employed to measure ocular surface deformation. An improved signal-to-noise ratio can be achieved by using a lock-in amplifier to modulate and confine the signal within a very narrow bandwidth and then filter the detected signal to eliminate most of the noise. Because the signal was constant, it was possible to diminish outside interference to a certain extent by averaging the measurement.

The material of the strain gauge was Constantan, an advanced copel alloy composed of 45 percent nickel and 55 percent copper. Constantan is the oldest and most widely accepted material for the use of strain gauges. The pros of the material include that it has a high electrical resistance to achieve a proper resistance for a small gauge length. Constantan foil also has a relatively low temperature induced strain that ranges from -30 to 193°C. It is considered a self-temperature compensating material. As composite of an advanced copel alloy, it has constant sensitivity across a wide range of strain. The use of Constantan foil as the grid material in these series of experiments resulted in a high correlation between IOP alterations and scleral deformations. Yet, a variety of other materials for strain gauge composition should be looked at.
to further improve design. Platinum in its pure form has the higher sensitivity when referring to the gauge factor. Composites of platinum with iridium and tungsten have also been shown to provide high sensitivity.

Designing a strain gauge with self temperature-compensation could improve the overall efficiency of a sclera-based sensor. Initial experiments were carried out with a quarter-bridge circuit and the use of only one strain gauge attached to the ocular surface. This was prone to thermal drift in certain instances depending on the setup. Thermal drift was corrected for but it seemed logical to employ a compensation to the temperature change by applying a half-bridge circuit, connecting two strain gauges together. The temperature effect was thus reduced by having a dummy gauge in the Wheatstone bridge circuit. It would be desirable to have a strain gauge that would provide self-temperature compensation when the temperature varies or the bridge circuit is not available. Although Constantan foil is classified as a self-temperature-compensating strain gauge, there are other materials that may prove less sensitive to thermal affects.

There are two types of strain gauges that can provide self-temperature-compensation. These include the selected melt gauge and the dual-element gauge. The selected-melt gauge has a very low thermal induced strain over a wide range of temperatures. The dual-element gauges uses two grids that provide varying thermal expansion properties, causing a net effect that cancels the affect of temperature drift.

Design specifications would entail that the strain gauge be constructed to smaller dimensions in order to be embedded in a contact lens carrier. The contact lens would have to be designed to properly fit under the upper lid on the bulbar conjunctiva. The present contact lens that was used for experimentation in Chapter 5 was chosen because of its diameter. The contact lens had a diameter of 13.8, the smallest of all silicone-hydrogel contact lenses. A smaller diameter was thought to provide a more comfortable fit, especially for patients who had never worn contact lenses before. Also, a wireless strain gauge design would be optimal. The present study used a pre-wired strain gauge. Several advances have been made to form wireless strain gauges. Other design specifications that would need to be addressed would be data conversion, bridge excitation, communication interface, and data storage.
Another possibility would be to use a capacity on-chip sensor device rather than a strain gauge. The proof-of-concept that scleral IOP can be measured and correlated to true IOP non-invasively and for extended periods of time allows for any type of micro sensing device to be used. The capacity on-chip sensor device is the most modern and technologically developed approach that employs the use of a pressure sensor system implant embedded on a single complementary metal-oxide-semiconductor (CMOS) chip. A CMOS chip is an integrated circuit that has high noise immunity and low static power consumption. In a CMOS inverter, one transistor of the pair is constantly off thus causing the series to obtain power only during switching between on and off stages. Also, CMOS do not produce heat. The design of the chip involves having a metal gate electrode placed on top of an oxide insulator that is in turn above a semiconductor material. Such a material would allow for miniature implants that have the power to transmit data for longer distances. Other advantages of such a material would allow for on-chip storage of data and a low signal-to-noise ratio.

Advances have been made in medical devices that have allowed for wireless communications. Several of these devices such as a heart monitor or a blood glucose monitor, have made recordings wirelessly and been sent to the patient’s medical practitioner. This allows for constant, hassle free monitoring and downloading into a common file that would be accessible not only to the patient but again to the clinical care practitioner. Radio frequency fields are generally used to transmit and power chips wirelessly and would be able to transmit the data digitally to a remote reader unit. A pressure dependent capacitor such as a micromechanical pressure sensor (MEMS) would have to be used. A MEMS sensor would be used to convert pressures into capacitance. Other possibility for wireless communications may use solar energy to collect device data. It would be of interest to design a wireless device that could use solar energy instead of obtaining power from an external device. This would imply that the contact lens carrier would have to be altered to collect solar energy. One way of doing this would be to increase the size of the contact lens so that it encompasses part of the cornea. By doing so, the energy in the form of light that would reach the ocular surface through the cornea could be collected.

The tradeoff between having a wireless device and the potential risk factors involved in using radio frequencies close to the eye to transmit information need to be addressed. A study conducted by Stang and colleagues (2009) examined the risk of developing uveal melanoma
The study reported an increased risk of uveal melanoma among mobile phone users and presented a case controlled study that assessed the association between wireless mobile devices and the risk of developing uveal cancer. The recruitment of 459 uveal melanoma case patients were performed at the University of Duisburg-Essen and matched 455 case patients with 827 population control subjects. One hundred and thirty three uveal melanoma patients were matched with 180 control subjects and another 187 uveal melanoma patients with 187 sibling control subjects. A questionnaire was distributed to determine the use of wireless mobile phone use. The study by Stang et al., (2009) estimated odd ratios and 95% confidence intervals of risk for uveal melanoma using conditional logistic regression. The authors concluded that risk of uveal melanoma was not associated with regular mobile phone use. The safety of a wireless ocular device would have to be further investigated.

The ability to use wireless communications to power and collect data would be beneficial. Yet, the ability to decipher the continuous IOP profiles of a patient still remains to be demonstrated. For instance, one challenge that can be seen with the collection of 24-hour IOP measurements is the ability to properly analyze and interpret the patterns. A patient would have to record their daily activities and events as a trial run to see if they can be associated with any changes or patterns recorded from the IOP monitor. For instance, if several fluctuations or spikes were to be recorded, would this be the true diurnal variation of a patient’s IOP or would this be representative of something else. If a patient were indicating that they were exercising, drinking water, or bending, any type of activity that has the potential to alter IOP, would help discriminate between true IOP properties or induced pressure changes. It is also assumed that a patients 24-hour IOP monitoring would show specific patterns pertaining to that individual. If these patterns were not reproducible or conserved over a 24-hour period, then longer recording times would have to be in place to decipher an individual’s pattern.

The ability of a wireless alarm attached to the sensor could alert patients and health care practitioners of high and low IOPs. This concept would help manage the disease by establishing when a patient’s IOP would spike or lessen. Again, providing insight into the diurnal variation of an individual’s IOP. Alarms implanted into the recording device could also warn the patient of missed medications or the urgency to seek medical attention if pressures are beyond normal. The wireless IOP monitoring device would have to have the capability of measuring low and high IOP ranges. If target IOPs are determined by the health care professional, then they could be set
as indicators in the device. If a target of 17 mmHg is reached, the patient and practitioner could be electronically notified, altering treatment regime.

There will be several challenges developing a wireless IOP monitoring device. Although there is no limitation in future design of such a device, difficulty incorporating all of these parameters and keeping size minimal, may prove difficult. Yet, with advancing technology, the introduction of nanostructures that are able to transmit information wirelessly, long distances, could help in properly designing such a device.

Another limitation that future designs may have is the biocompatibility of device. Several factors from embedding the strain gauge or sensor and coating applications have to be properly analyzed to confirm safety and biocompatibility for human application. There are several disciplines involved in biomaterial and biocompatibility studies that lead a path from a need to a manufactured medical device. The need to monitor IOP over extended periods of time has been identified. For years it has been implicated that current tonometric techniques are unable to continuously monitor IOP, especially at night when IOP may be at it’s highest. IOP needs to be measured throughout a 24-hour period. Whether that be every 5 minutes or every hour, it needs to be done during times when patients’ are not at the practitioners office and when they are sleeping. This thesis has provided proof-of-principle for the device design. A scleral IOP monitoring device can be implemented and can measure IOP correlated to real pressures. Of course device design has to be reorganized. A proof-of-concept employed an off-the-shelf strain gauge that was intended for uses other than ocular tissue testing. Material testing was also performed to determine bioreaction to the material in terms of cell inflammation. The contact lens carrier without the strain gauge was tested to confirm safety if worn on the sclera. The contact lens has already been FDA approved for overnight wear on the cornea, but since no other studies have looked at biocompatibility of wearing contact lenses overnight under the upper lid (conjunctiva), this was required. The strain gauge was not embedded in the contact lens material. Unfortunately, it was not possible at this point to test an embedded strain gauge sensor within the contact lens. The cost was too high and unattainable for a PhD project. Nevertheless, future directions would require that the strain gauge embedded in the contact lens be tested for mechanical properties, toxicology and biostability. The next step would be fabricating the device, sterilization and packaging. Device testing would include toxicology, in vitro interactions and animal testing. Pre-clinical or animal testing should be suggested to be employed in a
porcine model. The pig eye has many similarities to the human eye and is by far less expensive and offers few ethical problems than any other animal with close similarities to human eyes. The pig eye as a model for glaucoma has also been validated. Not only could device testing be conducted in such a model, but a glaucoma model could be also be developed. In other words, experimental glaucoma can be induced to further study the design of the scleral IOP monitoring device. Also, normal IOP measurements in the porcine eye mimic that found in the normal human eye, further establishing the correlation between a pig model and its significance for human application of a medical device. The only limitation in having a pig model would be set-up procedures. Because the pig is considered a large animal model, special consideration would have to be in place to determine how the strain gauge IOP monitoring device would stay in place for an extended period of time on the pig eye. Methods such as placing an animal cone for a 24-hour period on the animal to prevent device removal may be required. Future work in developing applications that would allow the scleral IOP monitoring device to adhere safely to the placement site would be optimal. Movement of the scleral IOP monitoring device largely depends on the design of the contact lens carrier. The experimentation in this thesis resorted to using an already approved FDA contact lens as a carrier material. The material of the contact lens was a hydrogel silicone (76% Lotrailcon A and 24% water) and proved biocompatible in terms of inflammatory response on the conjunctiva. Yet, the biocompatibility of the contact lens was not the major issue, but the diameter and size of it. Again, size may be a limiting factor in future designs of this device. A possible application would be to have a larger diameter covering the anterior sclera but without a central component of the contact lens so that the cornea is not covered. Again, experimentation needs to be performed on several contact lens carrier design options that would prevent movement of the device during recording. Future considerations may involve the use of a biogel to adhere the strain gauge solely to the ocular surface without the use of a contact lens carrier.

Pre-clinical studies from the pig model would have to illustrate that the device is not only able to recognize changes in scleral deformation in response to IOP manipulations, but also complies with safety and causes no inflammatory risk when worn over a 24-hour period.

The future success of this device depends on cost. Not only cost incurred to develop the device but cost for the patient. Initially, it was thought that if this device would be developed to a point of clinical use, that it could be employed in almost all patients. This would give more
information on IOP and how it is affected by other ocular diseases. It would also be used as a monitoring device for those individuals who are glaucoma suspects. Yet, having every patient wear a scleral IOP monitoring device may not be practical from a cost perspective. It is known that glaucoma care is costly with thousands of dollars being spent per person per year for therapies. However, the use of wearing an IOP monitoring device may have implications of targeting appropriate medicinal therapies for patients, meaning that less costs would be spent in trying various therapies that are not known whether the are appropriately decreasing IOP or not. Introducing such a device to the market would also require ethical considerations when partnering with industrial support. Since the company would fund much of the biomaterial research and own proprietary biomaterials, the needs of the patient must be balanced with the financial goals of the company. The need for this technology is crucial for further understanding IOP and its circadian nature. The future development of such a device would provide individualized therapy to patients with glaucoma and prevent individuals’ from developing glaucoma if properly used. Yet, the realization that such a device would be fabricated in the near future is far from reality. Several components of design and fabrication, biomaterial testing and preclinical and clinical testing need to be conducted. These components all take a considerable amount of time and money. The development of a medical device requires strict protocols to ensure safety and biocompatibility.

The development of a scleral IOP monitoring device, if properly designed and fabricated, may cause a paradigm shift in glaucoma care. A device that can provide information on IOP for an extended period of time during night and day would provide individualized patient therapy and new possibilities for glaucoma care and management.
References


Sherman D, Burkat CN, Lemke BN. Orbital anatomy and its clinical applications. Duane’s Ophthalmology, Eds. Tasman W, Jaeger EA. Lippincot Williams & Wilkins.


