INTRODUCTION

The ocular disease glaucoma is the second most common source of blindness worldwide. Gradual, irreversible vision loss is a result of nerve damage in the optic nerve head; a collagen structure that provides support for the optic nerve and blood vessels that enter the posterior of the eye [1]. Glaucoma is clinically known to be correlated with high levels of intraocular pressure from the fluid within the eye; intraocular pressure [2]. The method in which intraocular pressure causes nerve damage has yet to be discovered.

The laboratory of ocular biomechanics aims to study the eye as a biomechanical structure as a means to understand and ultimately prevent glaucoma progression. An increase of intraocular pressure causes stresses and strains on the optic nerve head that could result in deformation of the collagen support structure [3]. As the structure of the optic nerve head is better defined, calculating the deformation becomes possible. This deformation provides insight to what blood vessels or nerves are most affected by pressure changes, as well as what characteristics would make an eye more or less susceptible to glaucoma.

The sclera is part of the collagen region that makes up the optic nerve head [1]. The collagen in the sclera is oriented in fiber bundles called crimp, termed because of its wave like appearance. Because collagen performs well under tension, the wavy bundles stretch under increasing pressures [3]. To measure the stretch of the crimp, the period of the wave is recorded similarly to how the period of a sine wave is measured. Tracking the length of the crimp period shows how the collagen is changing and therefore how the sclera is deforming.

OBJECTIVE

To quantify the mechanical structure of the sclera, crimp period of a sheep eye can be measured using a standard technique within an imaging software. These measurements will ultimately give repeatable and reproducible results showing trends in crimp period change with regard to variously applied pressures mimicking intraocular pressure.

Besides qualitative observations the crimp measurements can be used for a number of statistical tests. To obtain additional information regarding characteristics of the eye, crimp measurements from one applied pressure can be used to statistically test the similarity of paired eyes. The test indicates whether the mean crimp periods of eyes from one sheep are more similar than two eyes from separate sheep or if paired eyes are irrelevant when considering crimp period.

HYPOTHESIS

It is reasonable to hypothesize that the crimp period in sheep eyes will increase with increasing intraocular pressure. As the pressure of the eye increases, the scleral deformation increases when the collagen stretches. It is likely that the period will get longer when the fiber bundle wave is lengthened.

Often individuals that develop glaucoma in one eye will develop glaucoma in the other eye shortly after. For this reason it is practical to hypothesize that the eyes taken from a single sheep would be significantly more similar than two eyes taken from different sheep.

METHOD

Sheep eyes were pressurized and fixed by the lab and the optic nerve head was excised coronally. The nerve heads were then imaged using polarized light microscopy. The light was filtered at four different angles providing four separate images of each sample. The images were taken in close proximity to obtain sufficient amount of detail. Due to the magnification each image displayed approximately one ninth of the coronal section. Once the images were uploaded onto the computer they needed to be stitched and aligned properly before analysis. The first step involves taking the nine magnified views and stitching them together using an imaging software called Fiji. Within this software there are various ways to stitch the images utilizing the overlapping rejoins of each section. Once there was a complete coronal section for each of the four angles of filtered light, the images were aligned in a stack. Using transparency and other tools, landmarks of the optic nerve head were hand adjusted to be directly in line with each other. The four aligned images condensed into a stack were considered ready for analysis.

The analysis was additionally done in Fiji. On the images, crimp appears as light and dark adjacent, somewhat parallel bands; due to the changing orientation of the collagen fibers within the wave. Each section of the sclera in which crimp was present, the line tool was used to mark one line over three adjacent sections of one light and one dark band. Each mark represents three periods of crimp, however; for the analysis the measured distance of each mark was divided by three to obtain the length of one period. This method allows the amount of error to be distributed over three periods and therefore is more accurate. The number of measurements varied depending on how much crimp was present, however on average there were approximately one hundred per stack.

To make sure that the measurements were accurate within the study, the researchers performed a repeatability and a reproducibility test. To test repeatability each researcher marked the same image three separate times. The test was deemed repeatable if the standard deviation of the mean was below two microns. To test reproducibility three different researchers marked the same eight images. This method was deemed reproducible if the standard deviations of the mean for all of the images were below five microns. Once the standard deviation thresholds were obtained, crimp measurements were completed for multiple sheep eyes over pressures ranging from 5mmHg to 50mmHg.
To compare crimp periods within a sheep and between sheep three sets of right and left eyes were selected from the previous data for study. Each of these eyes were subjected to the same pressure of 5mmHg. Each eye was compared to its respective match as well as to each of the other eyes in the study. A linear mixed effect model with an alpha of 0.05 was used to perform a statistical analysis to test for crimp period similarities.

RESULTS

The results showed that the markings were repeatable within researchers in the lab, and they were reproducible among researchers in the lab. These findings show that the crimp measurements are going to be accurate and standard over many images.

The measurements from all the images were recorded and organized, and crimp period was calculated. A trend in period length was present with respect to increases in applied pressure. In general there were fewer regions of crimp present and therefore a smaller number of markings made as the applied pressures were increased. Opposite of the original hypothesis, the results showed that crimp decreases with increases in intraocular pressure.

The results of the crimp comparison within and among sheep showed to be statistically significant. It was found that the average crimp period within the studied sheep was approximately three times more similar than the average crimp period among the different sheep.

DISCUSSION

Through the repeatability and reproducibility tests we were able to confirm that the method of marking chosen was accurate within ourselves and among the researchers in the lab. This is crucial to validating the observed results from any impending tests involving crimp period.

The results of the changing crimp period with changing pressure opposed the original hypothesis. Decreasing crimp period with increasing pressure initially seems counterintuitive. The collagen is stretching under tension and if it is oriented in wavy bundles it would seem as if the period naturally would increase. There is another factor present when considering what is happening to the crimp period. If the collagen stretches to its maximum then the collagen becomes a straight line and no longer appears as a wave. The lack of wave pattern is not visible on the scans and therefore there is no pattern to mark. Simply stated, at very high pressures the crimp stretches so much that it ‘disappears’. This theory is consistent with how the number of crimp markings decreases from over one hundred to less than one hundred as pressure is increases.

The results of the crimp comparison test aligned with the original hypothesis. Eyes from one sheep are closer in size and shape than a random sample so it seemed likely that the crimp periods would also be more similar. This test showed that consistency within a sheep accounted for approximately three times the similarity in crimp period. This is clinically relevant due to how glaucoma progress in a patient. Most patients that start exhibiting symptoms in one eye are likely to develop glaucoma in the other eye. From performing this statistical analysis, it is known that crimp period is a link between eyes in an individual and has the potential to link the effects of glaucoma onset.

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REFERENCES

