Collagen Fiber Waviness and Conformity: Are They Independent or Related?

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Introduction

Glaucoma is a condition that causes gradual, irreversible vision loss that can eventually progress to complete blindness. The main risk factor for the development and advancement of glaucoma is increased intraocular pressure (IOP) [1]. However, different eyes can have distinct levels of susceptibility to glaucoma at the same IOPs [1,2]. This biomechanical inconsistency is due to a natural, biological variability. The geometry and structure of tissue is a heavily studied source of this variability but tissue anatomy and composition also contribute. Little is known about how the latter specifically affects mechanical behavior other than it can mainly be attributed to collagen, the main load bearing component of soft tissue [2].

At the single fiber level, collagen displays an oscillating pattern known as crimp. When tensile stress is applied to a collagen fiber, the fiber straightens first and then bears the load abruptly. On the other hand, in a bundle of many fibers, each with unique crimp, each fiber will straighten at a different time and the bundle will bear the load gradually. Because of this behavior, knowing the characteristics of crimp for each fiber will allow us to predict how far each one can be pulled before it stiffens and then predict how the entire bundle will react to stress [3].

Waviness is one such characteristic of crimp. Defined as the standard deviation of the angles of a crimped fiber, waviness is a value the Laboratory of Ocular Biomechanics has already been able to quantify.

An equally important characteristic of collagen is how the bundles organize themselves. Basic physics states that the direction of the bundles affects how the tissue will respond to a force but direction is also important in how bundles interact with each other. Because of this, the characteristic, conformity is defined as the width of stacked bundles that have the same orientation. Conformity has not yet been quantified.

The overall goal of the lab is to quantify the collagen structure in the eye to understand how a specific IOP would affect a specific eye and how this relates to glaucoma. Our specific approach is to acquire sheep eyes from a butcher shop and section them axially. These 30 sections are then imaged with polarized light microscopy, a method of imaging that offers high enough resolution to observe fiber orientation on a micron scale.

Objective

The objective of my specific project was to determine if the stacking behavior behind conformity was attributed to how wavy the fibers were by testing the relationship between waviness and conformity.

Hypothesis/Success Criteria

I hypothesized that waviness and conformity were related. The success criterion for confirming this hypothesis was based on a statistical assessment, the Spearman test. To declare that the conformity and waviness were related, the resulting p-value needed to be less than our alpha (0.0001).

Our objective of determining that relationship was considered successfully met when a transformation of the conformity and waviness data met two other criteria based on statistical tests; the Linear Mixed Effect (LME) Models p-value had to be less than our alpha and the Akaike Information Criterion (AIC) had to be the lowest of all the transformations.

Methods

We began this process by acquiring the polarized light microscopy images described in the background. Two people at the lab and I then conducted a Repeatability Reproducibility (R&R) study on the lab’s proposed method of measuring conformity. This consisted of each person marking the width of approximately 800 predetermined stacks of collagen bundles 3 separate times using the image processing program, FIJI. For each of these predetermined stacks, the standard deviation of the lengths was calculated per person and across the three of us. The method was considered repeatable if all the standard deviations per person were less than 2 microns (5 pixels). The method was considered reproducible if all the standard deviations of all 9 measurements were less than 5 microns (9 pixels).

After confirming the R&R of the method, one student and I used it to gather conformity data. We collected 2,296 measurements from images from 3 different sheep eyes across 17 different regions of the eye.

The waviness from each conformity measurement was calculated and compiled to be statistically analyzed using R, a statistical language. First I completed a Spearman test that calculated a p-value using the t-distribution as well as a coefficient denoted as ρ (rho) that indicates how well the relationship between two variables can be described using a function that is entirely increasing or entirely decreasing [4].

Next, I calculated the squared, square root, cubed root, base e exponential, and base 10 exponential transformations of the conformity data. The LME of each transformation was calculated. LME is a linear model that accounts for data that came from various sources that can display the same trend with different ranges of data. Our example’s various sources, called random effects, are the 3 eyes and 17 sections that our data came from [5].

Finally, I extracted the AIC for each of the remaining transformations. The AIC is a measure of relative quality of statistical models. It offers an estimate of the information lost...
when the model represents the data. It balances how well the model fits the data and how complex the model is and only holds significance when compared between transformations of the same data to find best model [6].

RESULTS

The Spearman test calculated a p-value less than 2.2*10^-16. This is well below our alpha of 0.0001, indicating that conformity and waviness are significantly related. The p value produced was equal to 0.435. Because this value is positive, conformity and waviness are directly related. Consequently, we calculated the LME of transformations with positive relationships only, the results of which are listed in Table 1.

| TABLE I  |
|-----------------|-----------------|-------------------|
| **Waviness vs Conformity Transformation Results** | **LME p-value** | **AIC** |
| Conformity      | <0.0001         | 4679.068          |
| Conformity^2    | <0.0001         | 9878.928          |
| Conformity^1/2  | <0.0001         | 1794.607          |
| Conformity^1/3  | <0.0001         | 631.5635          |
| e^Conformity    | >0.0001         |                   |
| 10^Conformity   | >0.0001         |                   |

This step eliminated the base e exponential, and base 10 exponential transformation as potential relationships because their LME p-values were larger than our alpha. Finally, we extracted the AIC from the remaining transformations, the results of which are also displayed in Table 1. The cubed root of conformity had the lowest value, identifying it as the model with the most quality.

DISCUSSION

The Spearman test confirmed that conformity and waviness were related and determined the relationship was positive. The LME model narrowed down the possible relationships and the AIC allowed us to confidently choose the cubed root of conformity as the transformation that represented the data with the most quality. This relationship is shown in Fig. 1.

![Figure 1. Waviness vs the cubed root of conformity with line of best fit in blue](image)

Knowing that waviness increases a great deal when conformity increases brings about the question “why?” and researching the reason behind this relationship becomes our next step. The answer will hopefully be a statement about the behavior of collagen. The behavior will help us better understand how a specific IOP will affect a specific eye. Then we can relate that understanding back to the development of glaucoma.

This study was conducted with sheep eyes. The next step would be to confirm with human eyes and current technology shows promise in being able to do this in vivo. This research used tissue that had been sectioned and possibly altered in a way that changes our data from that of the original tissue. However, tools such as fiducial markers and image processing can counteract the possible error. The tissue in this study was fixed, a process also thought to possibly modify the results, and imaged with polarized light microscopy, a 2D imaging method. The next step concerning these approaches, respectively, would be to develop on a way to work with tissue that has not been fixed and to find a way to get a 3D image from the microscope. These adjustments in our methods would result in being able to gather the most accurate data to confirm the relationship found. Then the lab could go forth with predicting the biomechanical behavior of the eye with the most confidence. It is this assurance that will allow for the most precise understanding of why eyes respond differently to the same IOP and the best insight into the development of glaucoma.

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REFERENCES