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A SYSTEMATIC ANALYSIS CONFIRMED THAT MECHANICAL AND STRUCTURAL ANISOTROPIES DO NOT CONCUR IN 37% OF EQUATORIAL SCLERA SAMPLES

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INTRODUCTION

Many soft tissues are mechanically anisotropic, meaning that their stiffness varies by orientation [1]. Collagen fibers are the main load-bearing components of soft tissues, and therefore the macro-scale mechanical anisotropy arises from micro-scale structural anisotropy in the collagen fibers [2,3]. Thus, it is frequently assumed that mechanical and structural anisotropies concur [4]. For instance, it is common to use fiber orientation information obtained from techniques such as polarized light microscopy (PLM) or small angle light scattering to derive constitutive models in which the mechanical anisotropy essentially matches the structural anisotropy [5].

Recently, we measured the mechanical and structural anisotropies of equatorial sclera using standard equibiaxial testing and PLM techniques [2,5]. To our surprise, we found that the mechanical and structural anisotropies did not always concur. Although we only analyzed four samples from one eye, and the mismatch only occurred in half of them, we reasoned that a discrepancy between mechanical and structural anisotropies would be crucial to understand, even if it only occurs sporadically.

Our goal in this study was to systematically replicate our previous study, increasing the number of eyes and carefully checking each and every step to ensure that there were no mistakes or artifacts that could have produced the observed mismatch in anisotropies.

METHODS

Equibiaxial tensile testing. Five pig eyes were dissected to isolate equatorial sclera samples (11mm × 11mm) for each anatomical quadrant: nasal (N), superior(S), temporal (T), and inferior(I). A small cut was made on the top right corner of the samples to indicate the meridional direction. Mechanical anisotropy was measured using equibiaxial tensile testing with loading axes aligned with the equatorial and meridional directions to a maximum stress of 120 kPa.[6] (**Figure 1**) The specimen subjected to ten cycles of loading/unloading, and the data from tenth cycle was used for further analysis. The specimens were immersed in PBS during testing and switched to formalin afterward.

Polarized light microscopy (PLM). The samples were cryosectioned (30μm thick) and all sections imaged by PLM using an MVX 10 microscope [7]. The images were registered into stacks and collagen orientations computed for each pixel and then for the whole tissue (**Figure 2**).

Mechanical anisotropy was quantified by the ratio of the stiffness (the stress-strain curve slope at the point of full fiber recruitment) in the meridional and equatorial directions. Structural anisotropy was quantified from the ratio of areas under the fiber distribution curves.

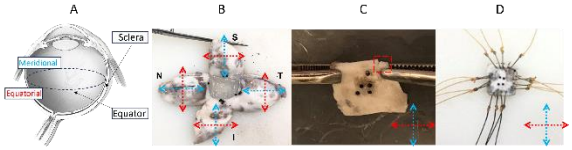

Figure 1: (A) Diagram of the eye with the location of the equator and the meridional and equatorial directions [6]. (B) Eye after dissection. (C) Excised quadrant with orientation mark. (D) Sample set for equibiaxial testing to measure mechanical anisotropy.

RESULTS

The equibiaxial test results show variable mechanical anisotropies between samples. Superior and inferior sectors had stiffer meridional directions (**Figure 3**). Nasal and

inferior sectors were closer to isotropic. The PLM measurements indicate substantial structural anisotropies, with a majority of the samples having the primary fiber alignment close to the meridional direction (**Figure 3**). Example cases with anisotropy match and mismatch are shown in **Figure 4**. One sample had problematic stretch tests and was excluded from further analysis. Overall, 7 of 19 samples (37%) had mismatched results between the mechanical and structural anisotropies (**Figure 5**).

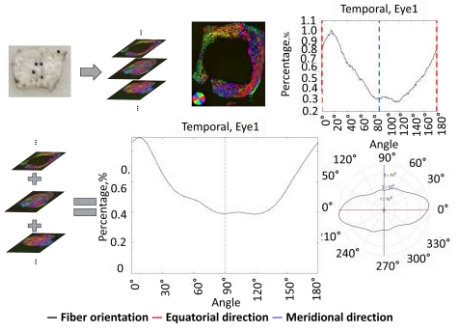

Figure 2: Structural anisotropy was measured from collagen orientation determined using PLM images of tissue cryosections. Shown are example images and orientation maps of a single section (top) and stack (bottom). This sample (T1) had clear anisotropy with fibers preferentially along the equatorial direction.

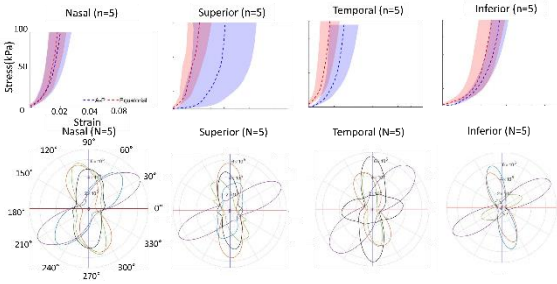

Figure 3: (top row) Arithmetic average (n=5) of 4 quadrants' strain at the same stress. Dashed curves represent the mean value of meridional and equatorial directions. The shaded regions are the standard error of the mean value. (bottom row) Polar plots of fiber orientations for all samples. Both tests show clear anisotropy and wide variations between samples.

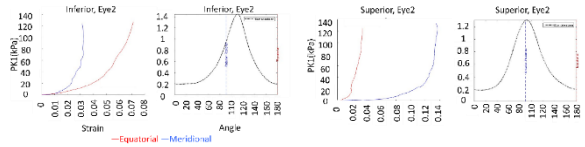

Figure 4: Matching (left pair) and mismatching (right pair) mechanical and structural anisotropies.

DISCUSSION

This study confirmed our previous finding that most equatorial sclera samples are mechanically and structurally anisotropic. Our observation that most equatorial sclera samples had collagen fibers preferentially aligned in the meridional direction is consistent with the literature [8]. Most importantly, our results confirmed that for 37% of the samples the mechanical and structural anisotropies do not concur.

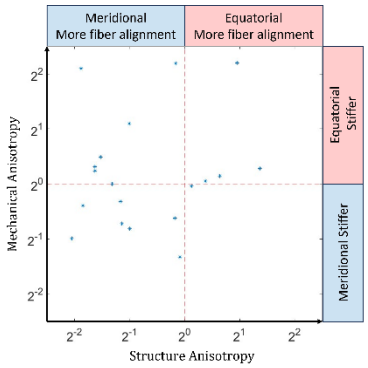

Figure 5. Scatter plot of R_{PLM} vs. R_{Mech}. The dashed line across the figure represents the mechanical and structure isotropic property. 7 samples are in mismatched regions. 12 samples are on the contrary.

The mismatch between anisotropies was not a surprise for this work. As we explained above, this study was motivated by the observation of the mismatch. In addition, in another study carried out independently by our group in collaboration with the Chen and Zhou labs in California we had observed anisotropy mismatches. Specifically, we had reported a mismatch between equatorial sclera mechanical and structural anisotropies. In those studies, however, mechanical anisotropies were not measured directly as we have done in this work. Tissue mechanics were instead inferred from measurements of wave propagation obtained from ultrasound and optical coherence tomography.

The causes underlying the mismatch between mechanical and structural anisotropies are not yet understood. This study cannot provide a definitive explanation for the mismatch phenomenon. But it is possible to speculate on some potential causes. One possible explanation could be differential collagen fiber crimp between orientations. This, however, seems unlikely as it would be discernible in the shape of the recruitment curve. It is also possible that the collagen fibers of the sclera vary in diameter [8], and thus in stiffness. It is also possible that there are directional variations in crosslinking [10], proteoglycan content or collagen type. Note that the PLM measurements are not perfect and do not account for the complex 3D nature of fibers that are interwoven. Fiber interweaving can also affect sample mechanics [11].

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