A subfibrillar deformation mechanism in corneal collagen that affords flexibility

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Purpose

To elucidate the hierarchical deformation mechanisms of the collagen network in the human cornea, at what strains they apply, and their timescales. To achieve this we devised an ambitious experiment that combined extensometry with small and wide angle X-ray scattering (SAXS/WAXS) and a simple scattering model.

Methods

In accordance with the tenets of the Declaration of Helsinki, 17 post-mortem human donor corneo-scleral disks aged between 21 and 90 years old were obtained from UK eye banks. Tissue was stored in culture medium at 37°C until 2 days prior to data collection, at which time it was supplemented with 15% dextran solution to reverse swelling effects.

A custom-built soft tissue extensometer compatible with control systems and stages at Diamond Light Source was built. The extensometer essentially comprised a piezo linear stage (Q-545, PI) and load cell (4.9 N Model 31, RDP), each attached to micromanipulator arms. Superior-inferior strips 3.5 mm across were adhered to the arms with cyanoacrylate. Distilled water was sprayed periodically on to the strips to maintain hydration.

2D and 3D digital image correlation (DIC) techniques were used to determine the macroscopic strain distribution on tensile strips during two different loading protocols. A paint speckle pattern (Fig. 1) was sprayed on to the strips to provide fiducial markers for tracking, and photographs were acquired using high performance cameras (Stingray F-504, Allied Vision). Instra (Dantec) and Matlab [1] were used to calculate strain fields.

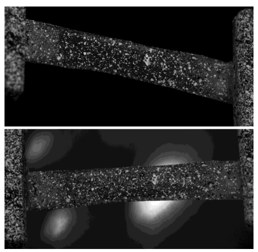

Figure 1: Strip of human cornea mounted on the extensometer and photographed from two angles. The two images are analysed to determine the 3D position of each point, and comparisons with other image pairs yields high resolution strain fields.

- Static protocol: Applying a tare load followed by static stretches of 1.4%, 2.8% and 5%. X-ray scatter images were acquired in lines along the centre of the strip at each strain increment after the sample equilibrated.
- Transient protocol: 5 cycles of ramped load to 500 kPa and unloading to 50 kPa, with each cycle taking approximately 1 minute. X-ray scatter images were acquired every 5 seconds from the specimen centre throughout the protocol.

X-ray images were analysed as previously described [2]. Supramolecular tilt was calculated by comparing fibrillar and molecular azimuthal distributions (Fig. 2) with a scattering model [3] (hardcopies supplied).

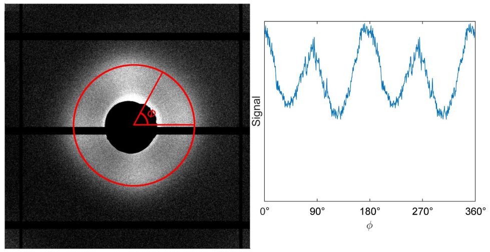

Figure 2 (Left): WAXS scatter pattern with intermolecular peak labelled. (Right): Azimuthal distribution of molecules based upon intermolecular peak distribution.

Results

DIC showed strain fields (Fig. 3) with peaks in the periphery and centre, and minima in the paracentre. Strain was heterogeneous on the anterior side, likely due to creasing associated with straightening corneal curvature. Creasing effects were not evident on the posterior side. The strain differential between centre and periphery was also seen in the transient experiment, (Fig. 4) however the periphery retained significant residual strain and deformed progressively less with each subsequent cycle.

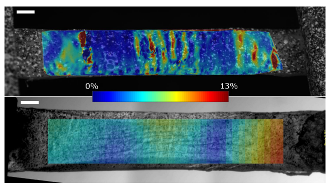

Figure 3: Strain distributions. (Top) Anterior side during transient experiment. (Bottom) Posterior side during static experiment. Bars 1 mm.

The azimuthal distributions of fibrils and molecules used to calculate average supramolecular tilts are shown in Figure 5. The scattering model maps the fibrillar distribution to the molecular distribution.

Changes in supramolecular tilt associated with specific static strains are shown in Table 1, along with the effective fibril stretch assuming the change in tilt is reflective of a spring-like deformation mechanism. At strains up to 2.8% the spring-like fibril stretch makes up the majority of the sample strain.

In the transient experiment, the supramolecular tilt changed in line with cycles of load (Figure 6), with the most pronounced change occurring in the first cycle. The effect diminished over subsequent loading cycles, suggesting that mechanisms with longer time constants take over. Static loading after preconditioning had a similar effect on supramolecular tilt to the normal static protocol.

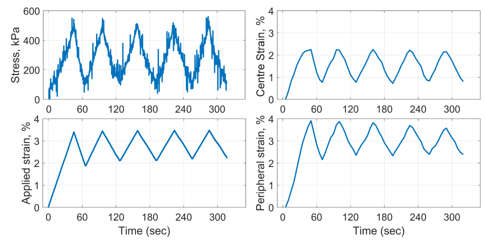

Figure 4: Comparison of applied stress and strain (left) with local strains calculated by DIC (right).

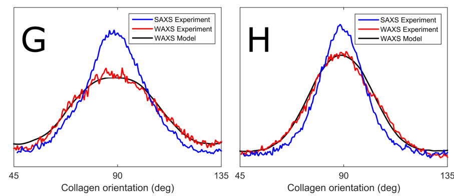

Figure 5: Azimuthal distributions of fibrils and molecules from a specimen under a tare load (left) and 5% static load (right). Scattering model data from Table 1 is overlaid.

Static strain	Molecular tilt	Fibril stretch
Tare	16°	0%
1.4%	14°	0.9%
2.8%	12°	1.8%
5%	11°	2.1%

Table 1: Molecular tilt and effective fibril stretch based upon spring-like deformation during the static loading experiment.

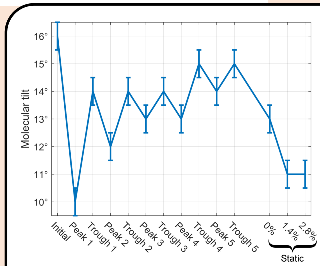

Figure 6: Molecular tilt during the transient experiment and at three equilibrium static strains following the cyclic loading pattern.

Conclusions

At small, physiologically-relevant loads (<2.8%) the dominant deformation mechanism in corneal collagen is a spring-like flex in supramolecular structure. Transient experiments show this mechanism has a short time constant, indicating it may enable collagen fibrils to elongate with minimal energy loss. This mechanism is likely to be fundamental in the normal mechanical function of the cornea in vivo, and can now be measured on a per-region basis.

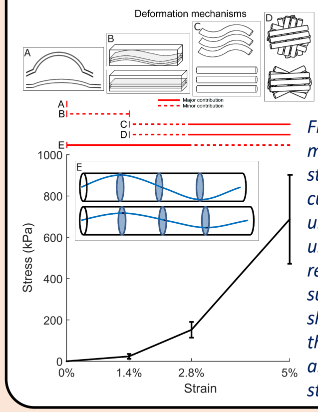

Figure 7: Deformation mechanisms (A) straightening of corneal curvature; (B) lamellar uncrimping; (C) fibrillar uncrimping; (D) fibril reorientation; (E) reducing supramolecular tilt. Spans show the strains at which the mechanisms apply, above the averaged stress-strain plot (bars SEM).

References

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[2] K.M. Meek, C. Boote, The use of X-ray scattering techniques to quantify the orientation and distribution of collagen in the corneal stroma. Prog. Retin. Eye Res. 28 (2009) 369–392.
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