Capturing the hierarchical response of human corneal collagen to controlled inflation

Session Number:
Posterboard Number:
Abstract Number:

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Purpose

To elucidate the hierarchical deformation mechanisms of human corneal collagen under controlled inflation of physiological magnitude. To achieve this we developed apparatus that allows the controlled inflation of corneoscleral discs in a synchrotron x-ray scattering beamline.

Methods

In accordance with the tenets of the Declaration of Helsinki, 6 post-mortem human donor corneoscleral discs 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 inflation device was built, comprising a 3D-printed pressure cell and annulus (Fig. 1) that secured the samples using O-rings and clamps, an electronic pressure regulator (QPV1, Equilibar) rated for pressures of 0-52 mmHg, a step-down regulator (RS) and flexible tubing. Samples were inflated using nitrogen. The I22 beamline at the Diamond Light Source synchrotron (Didcot, UK) allowed synchronous small- and wide-angle x-ray scattering (SAXS/WAXS) images to be acquired of the samples at pressures of 5 mmHg, 17.5 mmHg and 40 mmHg. The cell was mounted on a rotation stage to facilitate mapping. Distilled water was sprayed periodically on to the strips to maintain hydration, and samples were weighed before and after data acquisition to ensure hydration was maintained.

Figure 1. Left: Schematics of the inflation cell and annulus that secured the corneoscleral discs in position via O-rings and clamps. The back window was covered with BoPET (Mylar) to allow X-rays to pass through. Right: The inflation apparatus in use on the I22 beamline at the Diamond Light Source synchrotron.

X-ray images were analysed as previously described [1]. Variables of interest were the azimuthal distributions of collagen fibrils and tropocollagen molecules (Fig. 2), D-period, and the interfibrillar and intermolecular spacings.

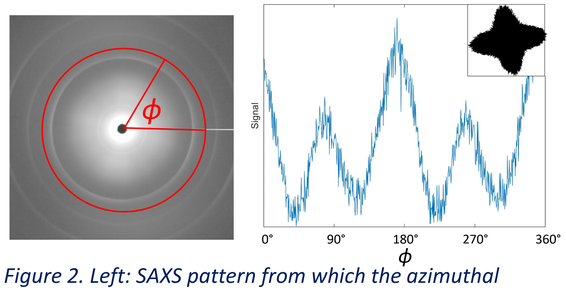

Figure 2. Left: SAXS pattern from which the azimuthal distribution of features are calculated. Right: Calculated azimuthal distribution of collagen fibrils. (Insert): Polar patch plot of azimuthal distribution.

Results

Scans were acquired along the nasotemporal meridian by rotating the pressure cell. Maps were built over an angular range of 80° centred approximately at the centre of the cornea. Images were acquired at intervals of 0.25°. This enabled the cornea, limbus and a small portion of the anterior sclera to be scanned with several scans in each region. Fig. 3 shows the azimuthal distribution of collagen fibrils at every 10th scan point at the 5 mmHg and 40 mmHg pressure points. The most significant changes are seen in the limbus.

These changes are seen more clearly in Fig. 4, which shows some collagen aligned circumferentially relative to the cornea reorientates into the radial direction.

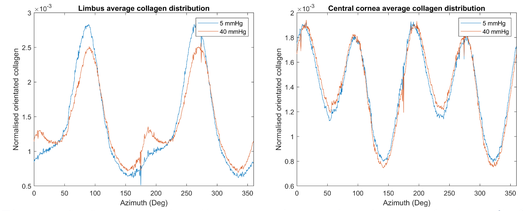

Figure 4. Averaged collagen orientation in the limbus (left) and the central cornea (right).

The D-period is a longitudinal measure of the axial stagger of tropocollagen molecules within the collagen fibril, and manifests on SAXS patterns as thin, circular peaks (Fig. 2, left). The D-period increases as fibrils stretch, allowing a direct measure of internal tissue strains. By measuring the D-period over specific angular ranges, directional strains in the collagen network can be measured. The size and radial position of meridional peaks (Fig. 5) provides information about the amount of collagen and D-period in that direction.

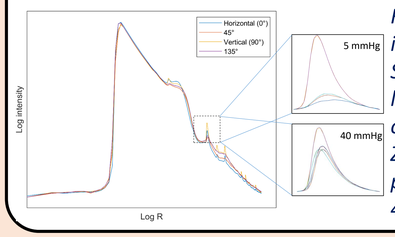

Figure 5. Left: Radial intensity plots from a SAXS pattern in the limbus, split into four orientations. Right: Zooms of a meridional peak at 5 mmHg and 40 mmHg IOP.

The directional measurements of D-period are plotted in Fig. 6, where a larger segment means a higher D-period in the segment direction (and therefore higher fibril strain). The largest changes are seen in the limbus, where radially aligned collagen significantly increases in strain at the higher pressure.

Conclusions

The changes in structure and D-period imply that the limbus experiences the largest strains associated with increases in IOP, as has been suggested previously, see Fig. 7.

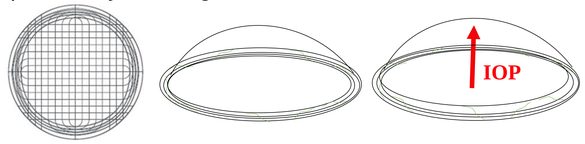

Figure 7. Left: Approximate arrangement of collagen fibrils across the human cornea. Mid and right: A model of a human cornea in equilibrium and following an increase in IOP, causing a stretch at the limbus. Adapted from [2].

An increase in D-period implies an elongation of collagen fibrils, which has recently been shown to occur under small loads due to the elastic *spring-like* straightening of helical supramolecular structures [3-4]. We are currently developing a model to calculate the extent of *tropocollagen spring* stretch across the cornea under inflation of physiological magnitude.

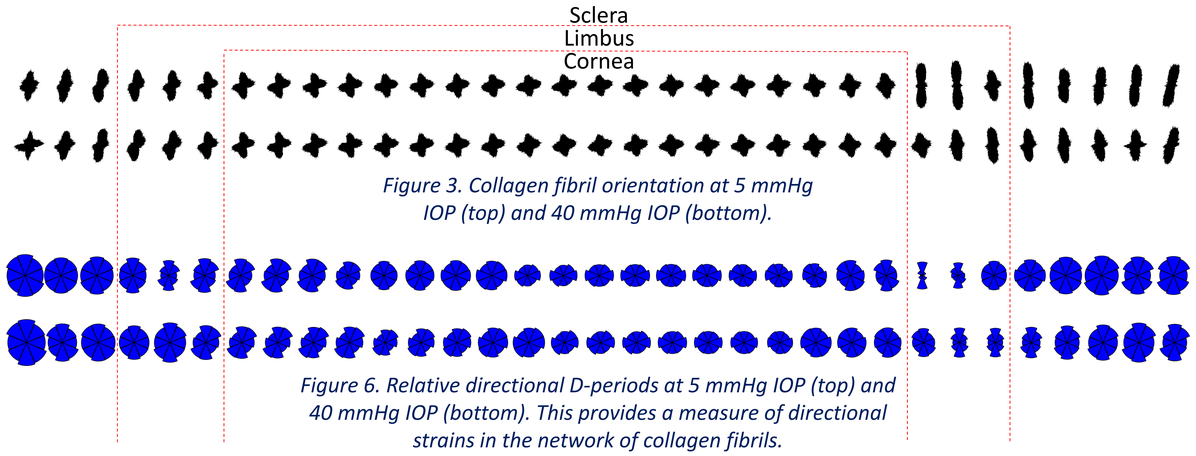

Figure 3. Collagen fibril orientation at 5 mmHg IOP (top) and 40 mmHg IOP (bottom).

Figure 6. Relative directional D-periods at 5 mmHg IOP (top) and 40 mmHg IOP (bottom). This provides a measure of directional strains in the network of collagen fibrils.

References

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[4] J.S. Bell, et al. Tropocollagen springs allow collagen fibrils to stretch elastically. Acta Biomater. 142 (2022) 185-193

This work was funded by Medical Research Council programme grants MR/K000837/1 and MR/S037829/1. We thank the staff of beamline I22 for their help in developing control software and scripting to co-ordinate inflation apparatus with X-ray scattering.