Capturing the hierarchical response of human corneal collagen to controlled inflation

James S. Bell¹, Sally Hayes¹, Siân M. Morgan¹, Elena Koudouna², Nick Terrill² Keith M. Meek¹
¹Cardiff University, UK; ²Diamond Light Source Ltd, UK

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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 corneal scleral discs in a synchrontron x-ray scattering beamline.

Methods

In accordance with the tenets of the Declaration of Helsinki, 6 post-mortem human donor corneal scleral 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 printed pressure cell and annulus (Fig. 1) that secured the samples using O-rings to allow X-rays to pass through. Right: The inflation apparatus in use on the I22 beamline at the Diamond Light Source synchrotron (Didcot, UK) allowed nitrogen. The I22 beamline at the Diamond Light Source Ltd, UK (RS) and flexible tubing. Samples were inflated using pressures of 5 mmHg, 17.5 mmHg and 40 mmHg.

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 10° scan point at the 5 mmHg and 40 mmHg pressure points. The most significant changes are seen in the limbus.

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.

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

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.

Conclusions

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.

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.

References


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