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Lamellar orientation in human cornea in relation to mechanical properties

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Abstract

We have applied wide-angle X-ray scattering to the human cornea in order to quantify the relative number of stromal collagen fibrils directed along the two preferred corneal lamellar directions: superior–inferior and nasal–temporal. The data suggest that, on average, the two directions are populated in equal proportion at the corneal centre. Here, approximately one-third of the fibrils throughout the stromal depth tend to lie within a 45° sector of the superior–inferior meridian, and similarly for the nasal–temporal direction. However, in some eyes we have measured significant differences between the two preferential fibril populations, with some corneas exhibiting as much as 25% more collagen in one direction than the other. Based on these findings, a mechanical model of the normal cornea may be envisaged, whereby the fibril tension in the underlying "background" of isotropically arranged collagen helps to balance the intraocular pressure; while the extra preferentially aligned fibrils take up the additional tensile stress along the superior–inferior and nasal–temporal meridians exerted by the rectus muscles and the orbicularis. It is possible that, through a direct impact on the elastic modulus of the tissue, an imbalance of superior–inferior and nasal–temporal fibrils in some eyes might affect corneal shape.

Keywords: Corneal stroma; Collagen fibrils; X-ray scattering; Biomechanics

1. Introduction

The human corneal stroma exhibits a layered structure comprising over 200 lamellae through its central thickness (Maurice, 1957). Each lamella consists of uniformly narrow collagen fibrils embedded in a hydrated matrix rich in proteoglycans, glycoproteins, salts, and keratocytes. Fibrils within a given lamella run approximately parallel, but subtend large angles with those in adjacent lamellae (Komai and Ushiki, 1991). Collagen fibrils reinforce biological materials (Jeronimidis and Vincent, 1984; Hukins and Aspden, 1985) and, because they are strongest axially, knowledge of their orientations within a particular tissue may be used to model its mechanical performance (Hukins, 1984). In the cornea, the relationship between fibril orientation and tissue mechanics is of considerable interest, since the mechanical performance of the cornea helps determine the shape of the tear film and thereby refractive status.

In 1938, Kokott was the first to suggest that collagen fibrils in the deeper layers of the stroma in the central cornea are not isotropically arranged, but rather adopt a preferential orientation along the superior–inferior and nasal–temporal corneal meridians (see Fig. 1). X-ray scattering studies later confirmed this, and indicated that the preferred orientation is more prevalent in the posterior half of the stroma (Meek et al., 1987). Notably, some have proposed that this preferential fibril

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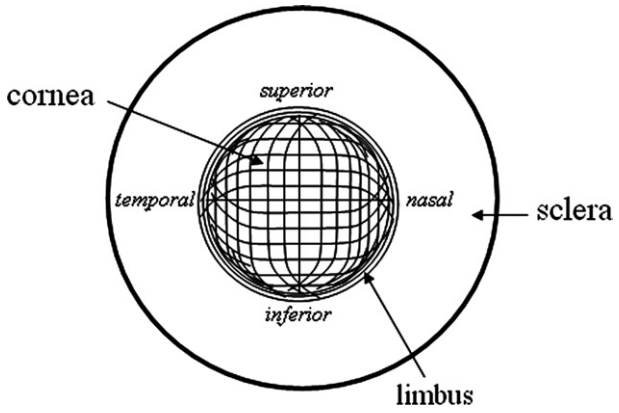

Fig. 1. Schematic showing the preferential orientation of collagen fibrils in the cornea of the human eye (right eye shown). The central cornea is characterized by a preponderance of fibrils arranged in the orthogonal superior–inferior and nasal–temporal directions. These fibrils are thought to bend at the periphery to coalesce with a circumferential annulus of fibrils where the cornea fuses with the sclera at the limbus.

orientation exists in order to take up the stress of the ocular rectus muscles along the corneal meridians (Daxer and Fratzl, 1997; Kokott, 1938). Whilst further quantitative analysis has indicated that, in total, around two-thirds of fibrils of the stroma tend to lie within 45° sectors of the superior–inferior and nasal–temporal directions (Daxer and Fratzl, 1997; Newton and Meek, 1998a), it has up to now remained unknown in what proportion each sector is populated. In order to resolve this question, we initiated a wide-angle X-ray scattering (WAXS) study.

2. Materials and methods

2.1. Specimens

Four time-expired healthy adult human corneas of various ages, obtained from the UK Corneal Transplant Service Eye Bank (Bristol, UK), were wrapped in cling film and stored frozen at −80 °C prior to the experiment. A further three corneas were obtained from the Ophthalmic Centre, Great Wall Hospital (Beijing, China), and stored in a 2.5% formalin solution. None of the donor corneas used had any previous history of refractive surgery. The 12 o'clock position of each cornea was identified with a limbal suture.

2.2. Data collection

WAXS experiments were performed on station 14.1 at the UK Synchrotron X-ray Source (Daresbury, UK), using an X-ray beam of wavelength 0.1488 nm which had a square 0.2 mm × 0.2 mm cross-section at the specimen. In order to minimise tissue dehydration during X-ray exposure, the corneas were placed in air-tight Perspex (Databank, UK) chambers with Mylar (Dupont-Teijin, UK) windows. X-rays were passed through the anterior corneal face parallel to the cornea's optical axis for 90 s per exposure, and the resulting WAXS patterns recorded on a Quantum 4R CCD detector (ADSC, Poway, CA) located 150 mm behind the specimen. A specimen translation stage (Newport, UK), interfaced with the X-ray camera shutter, allowed the specimen to be moved between exposures in the vertical and horizontal directions within the corneal plane. Nine diffraction patterns, from sampling positions forming a square 3 × 3 grid, were recorded from within the central 2 mm region of each of the seven corneas.

2.3. Data analysis

A typical WAXS pattern from one of the corneas is shown in Fig. 2A. It features a single reflection formed by interference between X-rays scattered by the regularly arranged collagen molecules lying near-axially within the stromal fibrils (Meek and Quantock, 2001). Each single stromal lamella will produce a pair of diffraction spots either side of a line representing the long axis of its constituent molecules/fibrils. It follows that corneal stroma featuring a completely homogeneous arrangement of lamellae would be expected to produce a circular X-ray reflection of uniform intensity around its circumference. However, as shown in Fig. 2A, diffraction patterns from the central human cornea generally exhibit four lobes of heightened intensity, arising from the orthogonal arrangement of preferentially aligned fibrils. By measuring the distribution of intensity around these peaks, we may quantify the extent of fibril alignment in the corneal stroma.

For each WAXS pattern, the normalised intensity profile (Fig. 2B) was obtained using Optimas 6.5 (MediaCybernetics, UK) image analysis software and Excel (Microsoft, UK) spreadsheets, a procedure which has been described in detail previously (Newton and Meek, 1998b). The intensity profile essentially gives the orientation distribution of the fibrils, once a 90° phase shift has been introduced to account for the fact that collagen molecules scatter X-rays in a plane perpendicular to their long axis. We then calculated the integral of the scatter intensity within a 45° sector of each of the superior and nasal directions, I_s and I_n in Fig. 2, and expressed these two values as fractions of the total scatter intensity summed through 180°. This gave a measure of the proportion of fibrils oriented along (or within 22.5° either side of) the two preferred corneal meridians, averaged throughout the stromal depth. Of course, since corneal WAXS patterns are by definition centro-symmetric, a similar analysis of the inferior and temporal directions would have yielded identical results.