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The Integration of the Corneal and Limbal Fibrils in the Human Eye

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ABSTRACT The precise orientation of the collagen fibrils in human cornea and sclera and the method by which these two areas fuse together at the limbus have never been determined, despite the importance of this information. From a consideration of the mechanics of the system, fibril orientation in the tissue has the potential to affect the curvature of the cornea so, by inference, refractive problems such as astigmatism involving an incorrect curvature of the cornea may be related to fibril orientation. The high intensity and small beam size of a synchrotron x-ray source has enabled us to study fibril orientation in post-mortem human cornea and sclera. Previously we have revealed two preferred directions of orientation in the cornea (Meek, K. M., T. Blamires, G. F. Elliot, T. Y. Gyi, and C. J. Nave. 1987. *Curr. Eye Res.* 6:841–846) and a circumcorneal annulus in the limbus (Newton, R. H., and K. M. Meek. 1998. *Invest. Ophthalmol. & Visual Sci.* 39: 1125–1134). Here we present the results of our investigation into the relationship between these two features. Our measurements indicate that the corneal fibrils oriented in the two preferred directions bend at the limbus to run circumferentially. On the basis of these results we propose a model as to how the human cornea and sclera fuse together.

INTRODUCTION

The cornea is the main refractive component of the eye, responsible for more than two-thirds of the total focusing, the rest being performed by the lens. The corneal stroma is made of ~200 lamellae, each roughly parallel to the surface of the eye. The lamellae are composed primarily of type I collagen fibrils packed in an ordered arrangement in a matrix rich in proteoglycans. Within each lamella the fibrils are all parallel (although of random polarity), but the fibrils of adjacent lamellae do not generally have the same orientation; the change in orientation of the fibrils between lamellae may be any angle between 0 and 180° (Maurice, 1969).

The sclera is also composed of collagen fibrils, but arranged in less ordered lamellae (Komai and Ushiki, 1991; Thale and Tillman, 1993). The precise orientation of the collagen fibrils in normal human cornea and sclera and exactly how these two areas fuse at the limbus are still unknown, despite the clinical importance of this information.

The orientation of fibrils in the cornea and sclera is important because, mechanically, fibril orientation has the potential to affect the corneal curvature, so by inference, refractive problems such as astigmatism involving an incorrect corneal curvature may be related to fibril orientation. Maurice (1988) reviews the mechanical properties of the cornea.

From mechanical considerations, the change in curvature between the cornea and sclera suggests circumferential fibrils. Maurice (1969) estimated that because of the change in curvature, circumferential tension at the limbus would be at least twice that in neighboring regions. Fischer (1933) reports that this zone is rich in circular fibers, and Ischreyt (1899) found strips of sclera taken parallel to the limbus had three times the tensile strength of meridional strips. Electron microscopy, although providing much information on the ultrastructure of the cornea (Komai and Ushiki, 1991; Thale and Tillman, 1993; Jakus, 1954; Haustein, 1983), has not been as successful in determining or quantifying large scale orientations. The high magnifications necessary to observe orientation are at the expense of seeing the tissue as a whole, so that it has not been possible to obtain information on orientation which is contiguous throughout the depth and across the tissue. X-ray diffraction, however, is well suited to providing quantitative fibril orientations.

X-ray fiber diffraction on post-mortem human tissue reveals the distribution of fibril orientations at the measurement point, integrated through the thickness of the tissue. The high intensity of a synchrotron x-ray source permits a small measurement spot size and a short exposure time, enabling a large number of locations in the tissue to be investigated at sufficient spatial resolution to map contiguous variations in the distribution of fibril orientations.

By using synchrotron x-ray diffraction, Meek et al. (1987) found two preferred directions in human corneal stroma, inferior-superior and medial-lateral. These preferred directions were more pronounced in the posterior than the anterior stroma. Toward the limbus there was one preferred direction tangential to the limbus. Measuring fibril orientations at a higher spatial resolution across the limbus at different points around the corneal circumference revealed a circumcorneal annulus of collagen fibrils (Newton and Meek, 1998). Here we present the results of our investigation into the relationship between the circumcorneal annulus and the two preferred directions in the cornea.

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MATERIALS AND METHODS

Samples

Time-expired whole excised human corneas with scleral rim attached were obtained from UK Corneal Transplant Service Eye Bank, Bristol. The orientation of the cornea with respect to the eye had been marked on excision by a suture in the scleral rim at the superior position. A left, 60-year-old cornea was used in this investigation and the results were verified on a right, 65-year-old cornea.

Synchrotron data collection

Data collection was carried out at station 7.2 of the CCLRC Daresbury Synchrotron Source using the Keele fiber camera: a wide angle camera that permits recording of the equatorial reflection from the collagen molecules within the fibrils (camera length = 0.142 m, x-ray wavelength = 0.1488 nm). A 0.2-mm collimator and an exposure time of 3 min were used. Samples were placed in an air-tight Perspex chamber with Mylar windows. With the scleral rim still attached a human cornea retains a uniform curvature and is robust to handle. The sample chamber was held in a carriage that could be translated between exposures in a horizontal direction by a stepper motor in steps of 0.2 mm.

The distribution of fibril orientations was measured at regular intervals along straight lines across the tissue. The anatomical positions of the measurement lines are shown in Fig. 1. Measurements were made at 0.2 mm intervals except within a 3.6 mm radius of the center of the cornea, where the spacing was 0.4 mm.

Data processing

Each diffraction pattern was normalized and the background, produced by scattering from nonfibrillar material, subtracted (Newton and Meek, 1998; Daxer and Fratzl, 1997). The fibrils have, in general, a 360° distribution so the equatorial reflection from the collagen molecules appears as a ring on the diffraction pattern. The distribution of fibril orientations was obtained from a measurement of the intensity around this diffraction ring, resulting in a graph of total scattering intensity versus angle of orientation for each point along the measurement line. The total scattering intensity is proportional to the total mass of collagen. These graphs were combined as contour plots to visualize the variation in the distribution of fibril orientations across the tissue. Additionally, the total scattering intensity was divided into its isotropic and nonisotropic components. At any measurement point, integrating through the thickness of the tissue, a proportion of the fibrils can be thought of as contributing to an isotropic orientation distribution, in which there is an equal number of fibrils/scattering intensity at all angles.

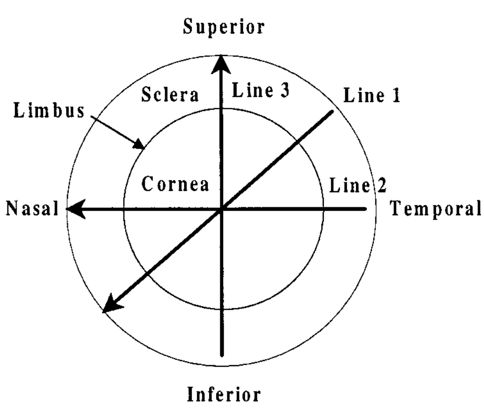

FIGURE 1 Schematic diagram of the sample cornea with scleral rim showing the positions of the measurement lines.

The remainder produce an orientation distribution which is nonisotropic (Newton and Meek, 1998).

The results are, in fact, an approximation to the distributions of fibril orientations because they are derived from the scattering from the collagen molecules, not the fibrils, and do not take into consideration the spread of angles of the collagen molecules about the fibril axis. The exact orientation of the collagen molecules within a single corneal or scleral fibril is complex and not accurately known, so at present it is not possible to correct the angular distributions. However, in other type 1 collagens, this angular spread is known to be small (Fraser et al., 1987), so the difference between the angular distributions of the collagen molecules and the angular distributions of the fibrils will also be small.

RESULTS

Fig. 2 shows the results of measurements along an oblique, superior-temporal to inferior-nasal, line. The total scattering intensity versus fibril orientation along the measurement line is given in Fig. 2 *a*. The isotropic and nonisotropic components of the scattering have been plotted separately in Fig. 2, *b* and *c*.

The average total scattering and the isotropic component of the scattering increase from the center of the cornea toward the periphery. This is a result of both the actual increase in the anterior-posterior thickness of the tissue toward the periphery and the curvature of the corneoscleral disk, which results in the x-ray beam passing through a greater thickness of tissue. At 4 mm from the center of the cornea the isotropic component is a factor 1.15 times that at the center. Crossing the limbus the isotropic component in the sclera is a factor 1.90 times that at the corneal center because of the further increase in the tissue thickness and an increase in volume density of collagen.

The orthogonal superior-inferior and nasal-temporal preferred directions of the cornea can be clearly seen in the graph of the nonisotropic component of the scattering intensity. The measurements shown in Fig. 2 are taken along a line running obliquely across the tissue so the preferred directions appear at 45 and 135° relative to the line. The random variation in the nonisotropic component of the scattering intensity across the cornea that can be seen in the preferred directions shown on Fig. 2 *c* probably reflects the known structure of the cornea: namely a superposition of criss-crossing, ribbonlike lamellae of up to 3 mm in breadth (Maurice, 1969). Within ±2 mm of the corneal center ~60% of the fibrils are oriented within the 45° sectors around the inferior-superior and nasal-temporal preferred directions, while 40% are oriented in the oblique sectors in between. This compares well with the average values of 66% and 34% obtained by Daxer and Fratzl (1997) using low-angle x-ray scattering on a larger sample of 17 normal human corneas. The difference can be accounted for by variability between individuals (Daxer and Fratzl, 1997).

The circumcorneal annulus produces a sharp peak in scattering intensity at an angle of 90° relative to the measurement line at a location in the sclera ~1 mm in from the limbus, where the limbus is taken to be situated at the center of the transition zone between the clear cornea and opaque sclera (Van Buskirk, 1989).

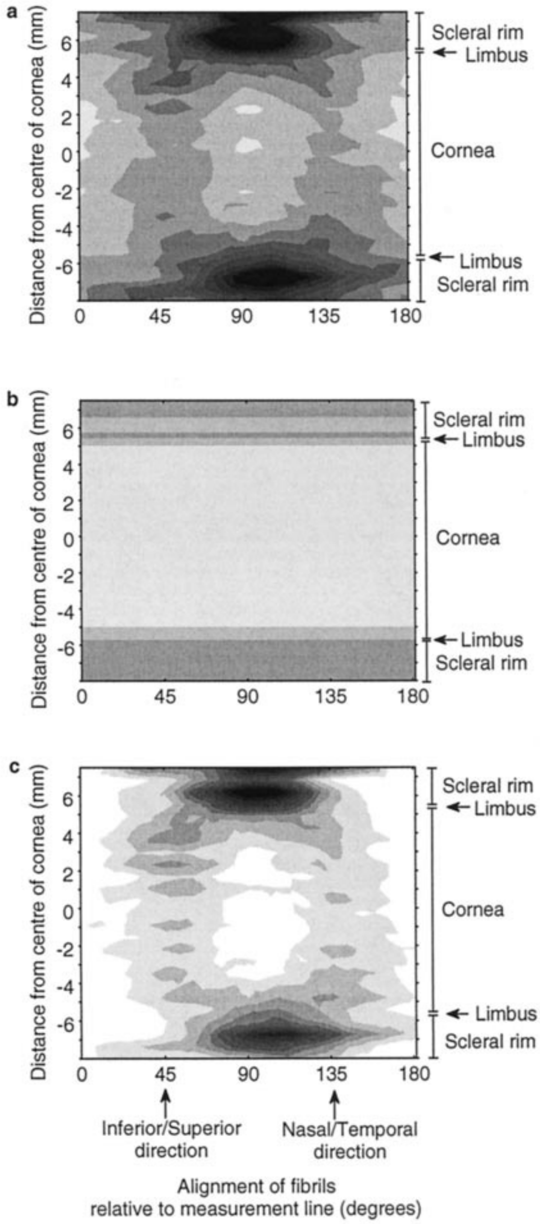

FIGURE 2 Measurements of the scattering intensity (equally spaced contours of arbitrary units) versus fibril orientation and distance along line 1. (*a*) Total scattering intensity; (*b*) isotropic component of the scattering intensity; (*c*) nonisotropic component of the scattering intensity.

It is apparent from Fig. 2 *c* that the corneal preferred directions remain orthogonal throughout the width of the cornea until ~1.5 mm from the limbus, when they bend through 45° in a space of 2.5 mm to merge with the peak produced by the circumcorneal annulus located 1 mm into the sclera. It is important to note that it is the distribution of fibril orientations at points along a straight line that have been measured. The measurements are not following the changes in alignment of individual fibrils/lamellae across the corneoscleral disk. A model for the behavior of the fibrils can, however, be deduced from measurements of the distributions of fibril orientations. Assuming that fibrils are continuous across the width of the cornea (Maurice, 1969) then the pattern of preferred directions in Fig. 2 *c* indicates that the fibrillar material aligned orthogonally at the center of the cornea must bend at the periphery to run circumferentially in the limbal zone.

The intensity of scattering from the preferred directions increases at the corneal periphery ~1.5 mm from the limbus. This may be because of an increase in aligned fibrillar material in the peripheral region of the cornea, reinforcing the fibrils from the center of the cornea. Alternatively, it may be the result of the bending of the fibrils causing an increase in fibril density.

The corneal preferred directions are known to be more pronounced in the posterior half of the cornea than in the anterior half (Meek et al., 1987). It is not known how the collagen fibrils of the circumcorneal annulus revealed in x-ray fiber diffraction patterns are distributed with depth through the limbal zone of the sclera. Some of the increase in the component of nonisotropic scattering is likely to be due to circumferential alignment in structures in the posterior limbus, such as the trabecular meshwork, but is not known in what proportion.

Fig. 3 *a* shows measurements along a temporal-nasal line. The slightly asymmetrical appearance of the graph is due to the measurements not following an exact diameter of the cornea. Again, the preferred directions remain mutually perpendicular until the most peripheral part of the cornea. The superior-inferior preferred direction (at 90° to the measurement line), constituting material running parallel to the tangent to the limbus, shows little change in orientation across the diameter of the cornea and merges with the peaks representing the circumcorneal annulus. The nasal-temporal preferred direction (at 0/180°) bends through 90°, beginning at a point 1.5 mm from either limbus. Extrapolating the trend of the preferred direction (see trend line, Fig. 3 *a*), it appears to have reached a circumferential orientation at a position corresponding to the limbus, rather than at a point in the sclera 1 mm behind the limbus, where the center of the peak representing the circumcorneal annulus is situated.

For an inferior-superior measurement line the nonisotropic material of the cornea (Fig. 3 *b*) shows a slightly different pattern of behavior. The material of the superior-inferior preferred direction (at 0/180°), oriented perpendicular to the tangent to the limbus, does not show a clear sign of bending 1.5 mm before the limbus. Instead, the preferred direction seems to terminate at the limbus, and in the zone 1.5 mm on either side of the limbus there is less material oriented between 90° and 180° to the measurement line than in Fig. 3 *a*. This may indicate that at the superior and inferior limbal zones, compared with the nasal and temporal, the fibrillar material bends with a smaller radius of curvature, too small to register with a spatial resolution of 0.2 mm. Further measurements are necessary in this area at higher spatial resolution than is currently available to us in order to verify whether this is in fact the case.

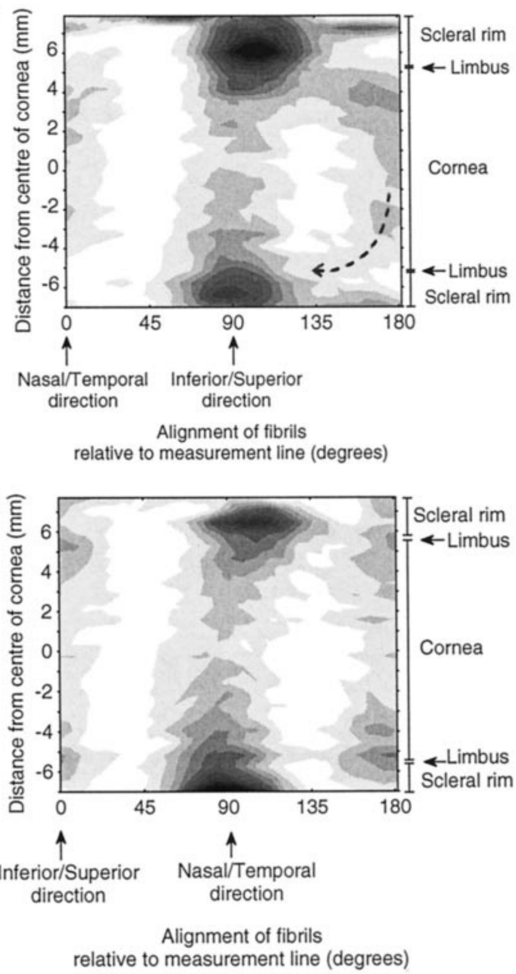

FIGURE 3 Measurements of nonisotropic scattering intensity (equally spaced contours of arbitrary units) versus fibril orientation and distance. (*a*) Distance along line 2. The dotted line on the graph indicates the trend of the nasal-temporal preferred direction in the limbal zone. (*b*) Distance along line 3.

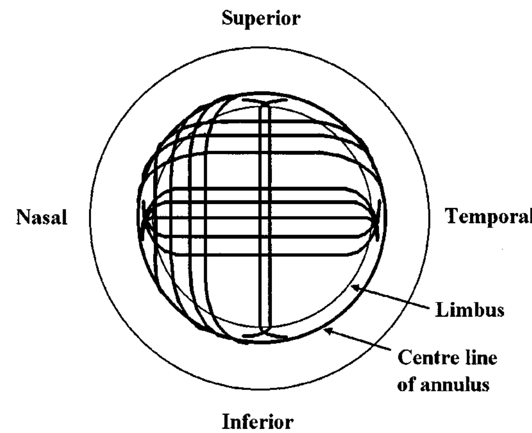

FIGURE 4 Schematic diagram showing the proposed model for fibril orientations based on the experimental measurements. The bold lines show the trends of the preferred directions of the fibrils in selected regions of the corneoscleral disk.

Fig. 4 shows a schematic diagram of the model, which best fits the experimental results, of the integration of the nonisotropic corneal fibrils with material in the limbus.

DISCUSSION

Synchrotron x-ray diffraction has enabled us to begin to unravel the fibrillar structure of the human cornea and sclera and propose a model of the manner in which they fuse together. We feel this is a first step in understanding the underlying structural, and hence mechanical, reasons for some refractive problems. An example of such a problem is surgically induced astigmatism. Incisions into the corneoscleral disk lead to changes in corneal curvature. This is used to correct astigmatism in astigmatic keratotomy, but can also be an unwanted side effect in other ocular surgeries. Surgically induced astigmatism can be a major problem in cataract surgery and keratoplasty. Cataract surgery requires an incision to be made into either the sclera, cornea, or limbus. The optimum incision site to minimize or eliminate surgically induced astigmatism is currently being investigated empirically (Kohnen, 1997). Anders et al. (1997) found that an incision in the sclera at a point 1 mm behind the limbus induced less astigmatism than a limbal incision. Our model may provide an explanation for this finding. A scleral incision tangential to the corneal circumference will penetrate the circumcorneal annulus parallel to the majority of the fibrils, and cause less damage than a limbal incision, which will cut perpendicularly through corneal fibrils that normally integrate the cornea with the annulus, before they have merged with the annulus. The same study (Anders et al., 1997) also found that an incision in the superior position induced more astigmatism than a temporal incision. It is not possible to say at present whether the differences we observed between the superior-inferior and the nasal-temporal measurement lines in Fig. 3 reflect genuine differences in the fibril alignment along these two diameters or are due to slight differences in the positioning of the two lines with respect to the cornea.

With more detailed spatial resolution and comprehensive coverage we hope to refine our understanding of the structure and provide further insights into refractive problems, both naturally occurring and surgically induced.

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