Collagen Fibril Orientation in the Human Corneal Stroma and Its Implication in Keratoconus

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Purpose. The kind and the degree of preferred collagen fibril orientation in normal human corneal stroma were investigated as important qualities of the cornea with respect to its mechanical properties and, hence, to refractive surgery. To determine whether this information is relevant to corneal disease, the authors investigated collagen fibril orientation in several corneas with keratoconus.

Methods. By means of low-angle x-ray scattering, 17 normal human corneas and four corneas of eyes with keratoconus were investigated.

Results. Collagen fibrils in the normal human corneal stroma showed two preferred orientations orthogonal to each other. These were the horizontal and the vertical directions. The authors defined a degree of orientation γ, determined to be $\gamma = 0.49 \pm 0.10$ (mean $\pm$ SD). This means that the excess of the preferentially oriented fibrils in relation to the total number of fibrils was approximately 49%. It follows from this value that approximately two thirds of the fibrils (66%) were within in a 45° sector ($\pm$22.5°) around the horizontal and vertical meridians, whereas approximately one third (34%) is oriented in the oblique sectors in between. No statistically significant variation of γ within a central 7 mm zone could be detected in normal corneas. The orthogonal arrangement of the collagen fibrils was, however, profoundly altered in keratoconus, in which nonorthogonal orientations were found inside the apical scar.

Conclusions. The normal human corneal stroma shows a considerable degree of structural anisotropy. It is characterized by two preferred collagen fibril orientations orthogonal to each other. Alteration of the regular orthogonal arrangement of the fibrils in keratoconus may be related to the biomechanical instability of the tissue. Invest Ophthalmol Vis Sci. 1997;38:121–129.

The human corneal stroma is composed of approximately 200 successively stacked lamellae of type I collagen fibrils. Within each lamella, the collagen fibrils run parallel to each other and show a regular interfibrillar spacing.[1–4] Orientation of the fibrils is constant within each lamella but varies throughout successive layers in a way that may depend on species, as well as other conditions.[5–8] Although the regular arrangement of the fibrils within each lamella is considered to be responsible for the transparency of the tissue,[1,9,10] the orientation of the successive fibril layers throughout the entire cornea is an important factor determining the mechanical properties of the cornea.[11,12]

Although many local details of the corneal structure have been obtained by electron microscopic methods,[e.g.,5] information about typical structural characteristics of the entire cornea, such as preferred or random orientation of the layers, is obtained more readily by scattering techniques.[4,8,13] In particular, Meek et al[8] reported x-ray scattering patterns from normal human corneas, indicating preferred orientation of collagen fibrils along the horizontal and vertical meridians. Nevertheless, the collagen fibril orientation in the human corneal stroma has never been characterized quantitatively. Such quantitative information is essential for an understanding of the biomechanical properties of the cornea and, hence, for the optimization of refractive surgery.[14–16]

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In this study, we used x-ray scattering to determine quantitatively the distribution of collagen fibril orientation in 17 normal human corneas at five positions (central, nasal, inferior, temporal, superior) within a central 7 mm zone. For comparison, we investigated four corneas of eyes affected by keratoconus, both inside and outside the lesion. In these eyes with keratoconus, considerable changes were found in the preferred angles between the collagen fibrils.

MATERIALS AND METHODS

Specimens and Preparation

Seventeen human corneas with no apparent sign of corneal disease were removed within 5 hours of death, and four corneas of patients with keratoconus were obtained after penetrating keratoplasty. Corneas were marked at the anatomic 12 o'clock position with one stitch of a 10-0 nylon suture. All corneas were stored at −70°C until measurement on the low-angle x-ray apparatus was performed. In agreement with other studies,[4,17] preliminary experiments have shown that the freezing procedure had no detectable influence on the scattering patterns of the corneas. The 17 normal corneas were fully transparent, and no lesions could be detected on slit lamp examination immediately before and after explantation. Each of the four keratoconus corneas had typical scars. Donors (15 women and two men) ranged in age from 38 to 89 years (median, 71 years). The state of hydration was estimated during the x-ray scattering experiment using the position of the main maximum of the scattering pattern: The interfibrillar distance, d, was approximately the same in all samples (d = 59.1 ± 2.2 nm; mean ± SD), indicating a hydration only slightly smaller than normal.[4] The tenets of the Declaration of Helsinki were followed, and approval of the responsible institutional human experimentation committee was granted. Keratoconic corneas were taken after informed consent was obtained.

Apparatus and Measurement

As described elsewhere,[4] we used a 12 kW x-ray generator and a pinhole x-ray camera with a fixed distance of 100 cm from the sample to the detector. The detector was a two-dimensional position-sensitive proportional counter (Siemens AG, Karlsruhe, Germany). The x-ray beam (diameter, 0.5 mm) had a wavelength of λ = 1.54 Å and was directed parallel to the optical axis at various corneal positions. In the normal human corneas, the positions were the center, nasal, inferior, temporal, and superior margins of a central 7 mm zone. Measurements were performed without further preparation in a native state of the corneas at room temperature. For measurements, the hydrated corneas were fitted carefully into an air-tight container made of welded plastic and were mounted into a vacuum chamber of the x-ray scattering apparatus. This procedure leaves the corneal buttons at approximately the correct curvature. An optical window in the wall of the vacuum chamber permitted visual observation of the tissue during the experiment. Typically, x-ray spectra were collected for 30 minutes and were corrected for absorption and parasitic pinhole scattering. The apparatus was calibrated by measuring the Debye–Scherrer rings of Ag-Behenate.[18]

Analysis of X-Ray Scattering Patterns

Two-dimensional, small-angle, x-ray scattering patterns were collected as schematically shown in Figure 1. Examples for such two-dimensional patterns are shown in Figure 2. These two-dimensional spectra were described as functions $J(\theta,\phi)$ of the scattering angle θ and the rotation angle ϕ (for definition of the angles, see Fig. 1). In a first step, these spectra were used to obtain the curve $I(\theta)$ by summing all values of $J(\theta,\phi)$ inside a ring between θ and $\theta + d\theta$ (with ϕ varying from 0° to 360°). A typical result is shown in Figures 3A and 3B. The maximum in the region between θ = 0.35° and ϕ = 0.60° (shaded area, Fig. 3A) corresponds to the first subsidiary maximum visible in green in Figure 2 (red arrow), which, according to Worthington and Inouye,[19] was caused by the first maximum of the equatorial scattering from collagen and contains a contribution from the sharp meridional collagen reflections (that is, the sharp third-order reflection located at 0.41°). For cylindrical fibrils, the equatorial scattering is approximately the Bessel function $(2\ J_1(ka)/ka)^2$ multiplied by the interference function of the cylinder centers L(k), with $k = 4\pi/\lambda \sin(\theta/2)$. Although the peak should, in principle, be caused by collagen, the background scattering below would arise from matrix proteins other than collagen inside the corneal stroma. Indeed, because the maximum corresponds to the oscillation of the Bessel function, the scattering from collagen fibrils should become zero on both sides of the maximum in $I(\theta)$. The contribution from the interference function resulting from fibril packing L(k) has been shown to be approximately constant and proportional to the number of fibrils in the k-range beyond the first zero of the Bessel function.[19] Consequently, the value of $I(\theta)$ at these two mimima of the scattering intensity should be caused by the background from matrix proteins other than collagen. We have interpolated between these two points to obtain a reasonable estimate for the background, as shown in Figure 4. The fitted background function is shown as a solid line in Figures 3B and 4. It corresponds to a power law behavior with the related intensity proportional to θ^{κ}, where κ = −1.02 ± 0.072 (mean ± SD) (n = 67). Figure 5 shows the integrated scattering intensity $I(\theta)$ subtracted by the background function. It can be seen that the mini-

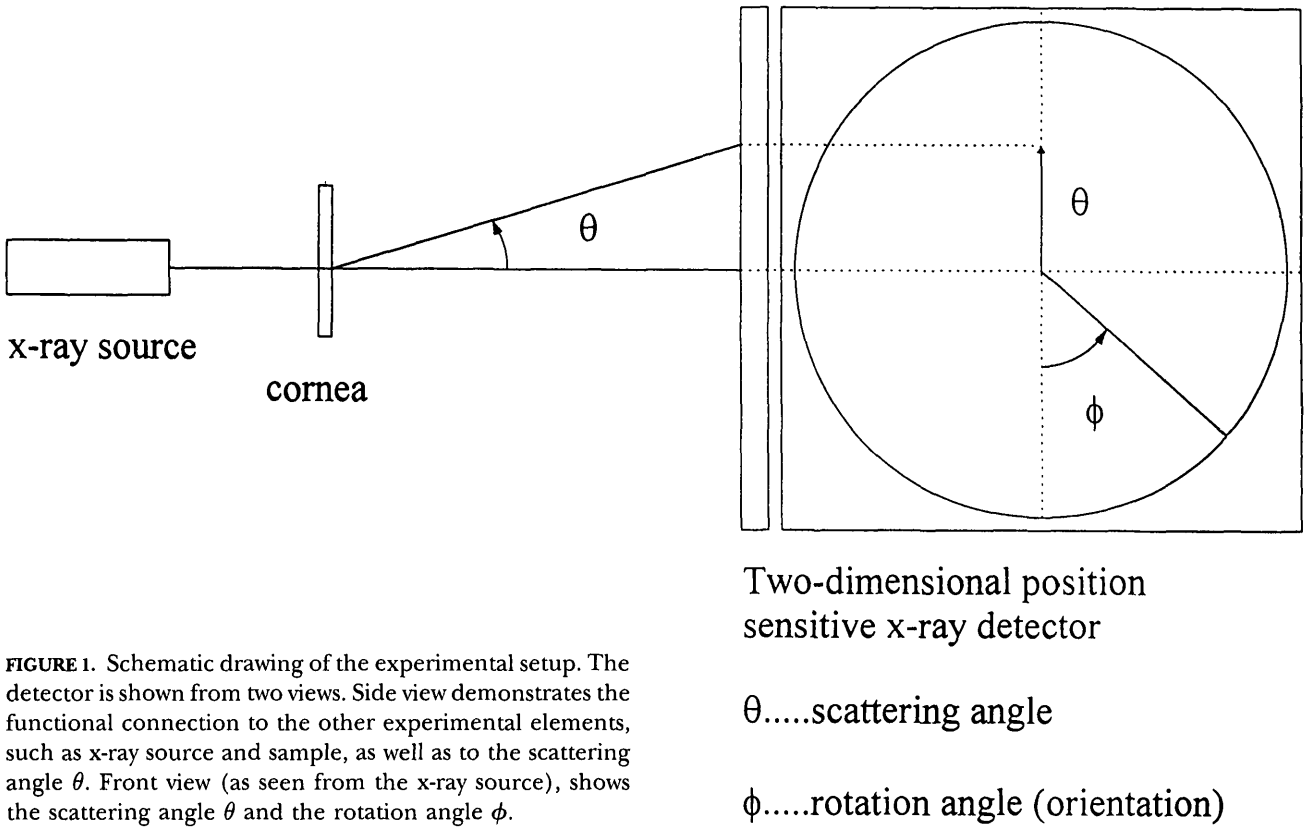

FIGURE 1. Schematic drawing of the experimental setup. The detector is shown from two views. Side view demonstrates the functional connection to the other experimental elements, such as x-ray source and sample, as well as to the scattering angle θ. Front view (as seen from the x-ray source), shows the scattering angle θ and the rotation angle ϕ.

mum at $\theta = 0.35°$ is entirely determined by the fibril transform, which is fitted (solid curve in Fig. 5) to the data by $(2\,J_1(ka)/ka)^2$ (see also above). The meridional scattering of the collagen fibrils caused by the axial packing of their molecules, indicated by the arrows labeled 3 and 5 in Figure 5, contributes as sharp peaks *within* the subsidiary maxima.

In a second step, a curve $I(\phi)$ was obtained by summing up all the values of $J(\theta,\phi)$ at a given ϕ, for θ between 0.35° and 0.60°, corresponding to the region of scattering angles of the first subsidiary maximum. Generally, the scattering intensity must fulfill the condition $I(\phi) = I(\phi + 180°)$. Because $I(\phi)$ is measured in fact for $0 \leq \phi \leq 360°$, we averaged $I(\phi)$ and $I(\phi + 180°)$ to improve the counting statistics and, therefore, the accuracy of the measurement. A typical result is shown in Figures 3C and 3D. The oscillation of the curve in these figures corresponds to the fact that the height of the first subsidiary maximum depends considerably on the rotation angle ϕ. This oscillatory part is seen clearly above a constant contribution, which is shown shaded in Figure 3D. $I(\phi)$ can be fitted by a constant (dashed line in Figure 3D) added to a sinus function (solid line in Figs. 3C and 3D). For each normal cornea, the height of the four maxima in Figures 3C and 3D was equal within the accuracy (counting statistics) of our x-ray measurements. This was not the case, however, for keratoconic corneas. Hence, the quantitative evaluation procedure described here was only applied to normal corneas.

In summary, the shaded areas in Figure 3 have the following significance:

Areas I_1 and I_3 are the total amount of scattering below the subsidiary maximum between $\theta = 0.35°$ and $\theta = 0.60°$, including the signal from all collagen (preferentially oriented or not) as well as from other matrix proteins.

Area I_2 is the scattering caused by noncollagenous proteins only, and area I_4 is the scattering caused by randomly oriented collagen as well as by noncollagenous proteins. It should be noted that I_3–I_4 (oscillations above the constant contribution in Fig. 3D) represents the excess of fibrils oriented in the preferred directions.

RESULTS

Structural Anisotropy in Normal Corneas

X-ray scattering spectra were obtained from 17 normal corneas at five locations each. An example is shown in Figure 2A, in which maxima in four orthogonal directions are visible. The four inner peaks (brown) are caused by interfibrillar spacing, whereas the four outer peaks (green) are related to oscillations of the fibril transform with a contribution from the third-order peak of the axial packing period of the collagen fibrils.[4,8,19] The orthogonal fourfold symmetry of the collagen-related peaks in the scattering patterns ob-

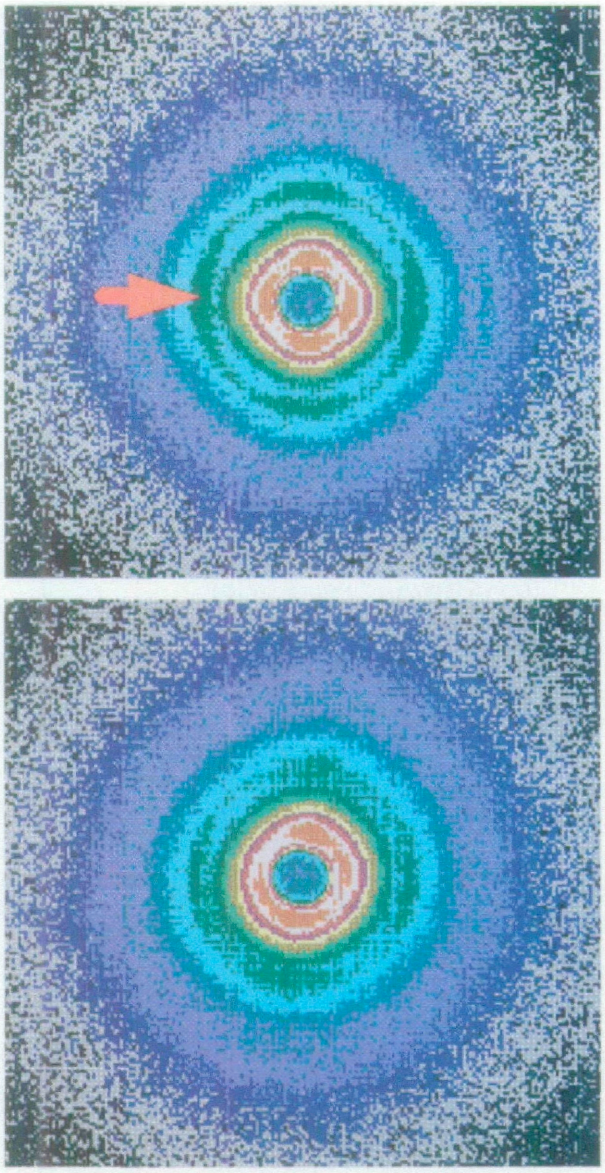

FIGURE 2. Two-dimensional x-ray scattering patterns of a normal cornea (*top*) and a keratoconic cornea in the apical scar (*bottom*). Inner reflections (brown) represent the scattering intensity caused by interference between the collagen fibrils, and their position (i.e., scattering angle measured as distance from the center) is related to the interfibrillar distance. The outer reflections (green, indicated by the red arrow) represent the first subsidiary maximum. It results from the scattering of both, the geometry of the individual collagen fibrils (fibril transform) (i.e. equatorial scattering), and the third-order reflection of the axial collagen period (i.e., meridional scattering). The presentation of the intensity distribution is based on a logarithmic scale. The normal cornea shows orthogonal fourfold symmetry (*top*); this symmetry is disturbed in the keratoconus (*bottom*).

tained from normal human corneas indicates preferred orientation of the collagen fibrils in two orthogonal directions. Moreover, within the counting statistics, all spectra for normal corneas were symmetric with respect to horizontal and vertical directions, implying that an equal amount of collagen fibrils were oriented vertically and horizontally. This remains true even we take into account that meridional (from the axial collagen period) and equatorial (from the fibril transform) scattering of the collagen fibrils contribute to the peaks of the first subsidiary maximum. This is because the main direction of the collagen fibril orientation found in the normal cornea are orthogonal; hence, the meridional and equatorial contributions superimpose at the same four positions. The four orthogonal peaks in Figure 2 are located on top of an intensity ring that resulted from collagen fibrils oriented in any direction. Although the preferred collagen fibril orientation in the vertical and horizontal directions is evident from the two-dimensional scattering pattern of normal human cornea (Fig. 2A), further analysis is required to obtain quantitative information regarding the amount of preferentially oriented collagen fibrils.

To quantify the degree of collagen fibril orientation (that is, the relation between the amount of preferentially oriented fibrils and the total amount of collagen fibrils), the two-dimensional scattering patterns (Fig. 2A) were integrated, as described above, yielding curves such as those in Figure 3 for each set of data. The ratio defined as $\alpha = (I_1 - I_2)/I_1$ [I_1 and I_2 indicating the areas of the shaded regions in Figs. 3A and 3B, respectively] represents the contribution of collagen (oriented as well as disordered) to the total scattering between $\theta = 0.35°$ to $0.60°$. The total scattering I_1 includes scattering from oriented and disordered collagen, as well as from other matrix proteins. A second ratio that may be defined is $\beta = (I_{3-14})/I_3$, which describes the amount of scattering from *oriented* collagen fibrils alone, relative to the total scattering between $\theta = 0.35°$ to $0.60°$ (I_3 and I_4 indicating the shaded areas in Fig. 3C and 3D, respectively). Although β can be thought as a lower bound for the degree of orientation of collagen fibrils, the "true" degree of orientation is defined as $\gamma = \beta/\alpha = (1 - I_4/I_3)/(1 - I_2/I_1)$. The ratio γ measures the amount of oriented collagen, relative to the total amount of collagen present in the corneal stroma. It is zero if all collagen is randomly oriented within the plane of the cornea and $\gamma = 1$ if all collagen is oriented superior to inferior and nasal to temporal.

Figure 6 and Table 1 show the degree of orientation γ for each cornea at each investigated position. γ varies widely and ranges from $\gamma = 0.29$ to $\gamma = 0.75$. In particular, we determined the following values for the degree of orientation γ at the different topographic positions:

γ (central) = 0.47 ± 0.09 (mean $\pm$ SD, $n = 15$);
γ (nasal) = 0.52 ± 0.11 ($n = 14$);
γ (inferior) = 0.51 ± 0.11 ($n = 13$);
γ (temporal) = 0.50 ± 0.08 ($n = 11$);

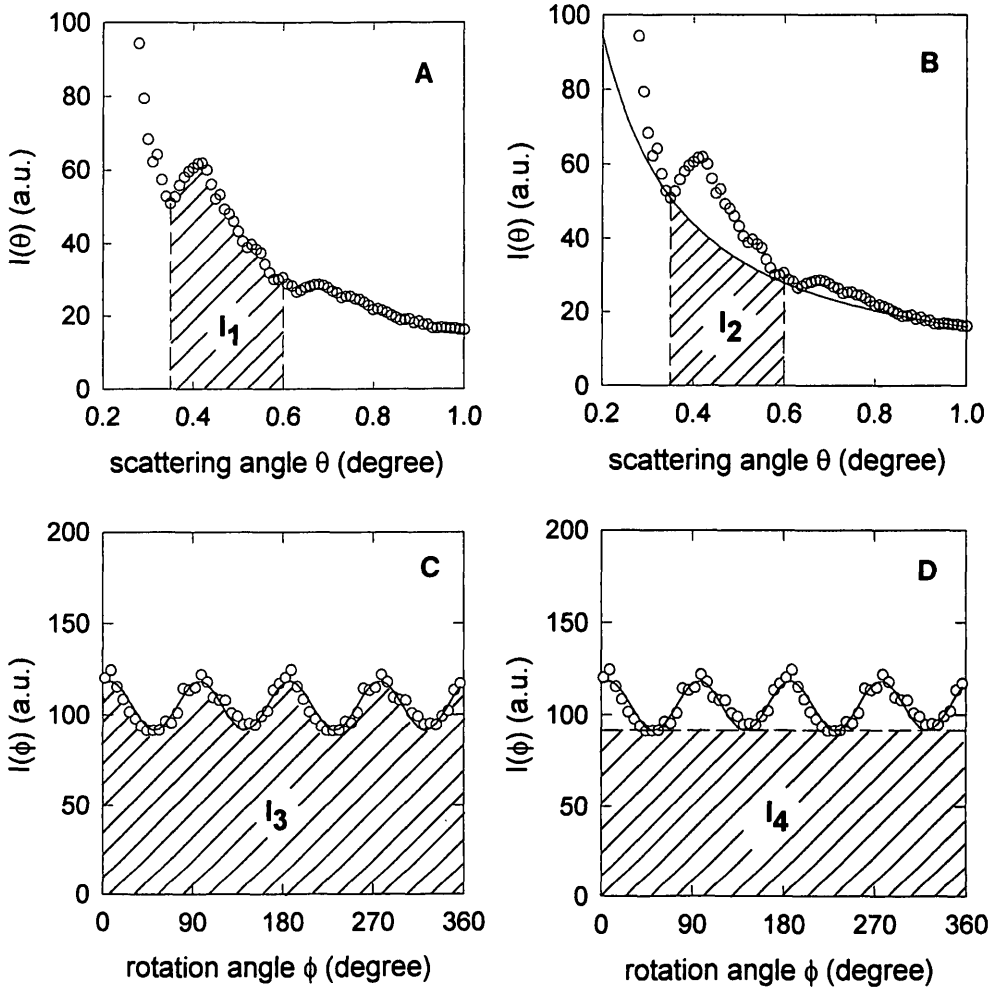

FIGURE 3. (A,B) Spherically integrated intensity I (θ). The maximum at 0.41° corresponds to the first subsidiary maximum. The shaded area I_1 represents the total integrated intensity. The shaded area I_2 represents the integrated intensity corresponding to the matrix elements other than collagen. (C,D) Integrated intensity of the first subsidiary maximum obtained by integration from θ = 0.35° to θ = 0.60°. As in I_1 in A, the shaded area I_3 represents the total integrated intensity. The shaded area L I_4 represents the integrated intensity caused by randomly oriented collagen fibrils, together with noncollagenous matrix elements.

γ (superior) = 0.45 ± 0.11 (n = 14);

γ (overall) = 0.49 ± 0.10 (n = 67).

The intraindividual variation of the degree of orientation γ (that is, the values of γ at different topographic positions within a central 7 mm zone of the same cornea) was not statistically significant.

Figure 7 shows γ averaged for each cornea over the investigated positions. The bars in Figure 7 indicate the standard deviations of the measurements. Although few corneas show considerable variation of γ between the different positions, it is apparent from Figure 7 and Table 1 that a low or high degree of collagen fibril orientation is primarily a property of the entire cornea and does not much depend on the location in the cornea.

The typical average value of $\gamma \approx 0.5$ indicates that there is an excess of fibrils of approximately 50% ordered vertically and horizontally over a background of fibrils, pointing with equal probability in any direction. Considering the sinusoidal variation of the intensity (Fig. 3D) and, therefore, of the number of fibrils, the probability that a fibril points in the direction Φ will be approximately $p(\Phi) = [1 + \gamma \cos(4\Phi)]/2\pi$, if Φ is expressed in radian ($0 \leq \phi < 2\pi$). Consequently, the total proportion of fibrils pointing away from the vertical and the horizontal by more than $\pi/8$ radians (= 22.5°) will be $4\int_{\pi/8}^{3\pi/8} p(\Phi)d\Phi = 1/2 - \gamma/\pi$. With the experimentally determined value for γ = 0.49, this means that in normal human corneas, only approximately one third (34%) of the collagen fibrils point away from the vertical or the horizontal by more than 22.5°. In other words, approximately two thirds (66%) of the fibrils are oriented in a 45° sector around the vertical and horizontal meridians, whereas only one third (34%) are oriented in the oblique sectors in between.

Keratoconus

In contrast to normal corneas, the situation in corneas obtained from keratoconic eyes appears more complex. Figure 2B shows an example of a two-dimensional x-ray scattering pattern from the scarred region in keratoconus.

The orthogonality of the collagen-related peaks, which is a characteristic quality of normal human corneas (see Figs. 2A and 3) has changed to an irregular scattering pattern in the center of the scarred region

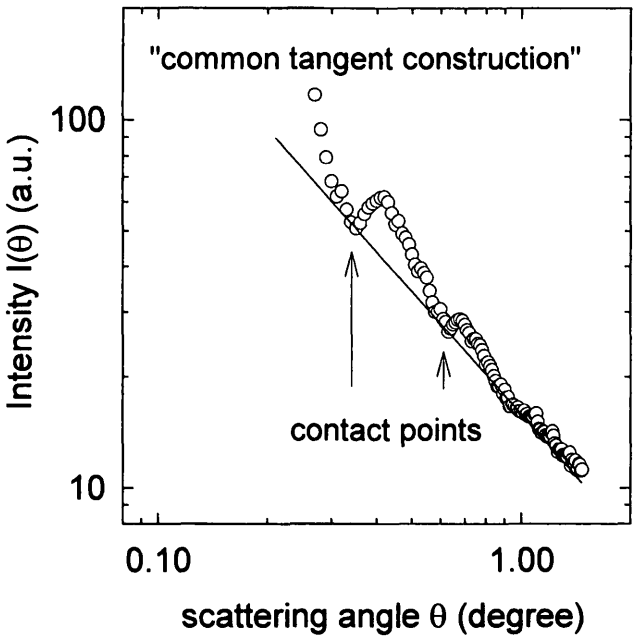

FIGURE 4. (*circles*) I(θ) in a double logarithmic plot. (*solid line*) This shows how a background under the peak at 0.41° was interpolated. It is defined by the two contact points on both sides of the maximum. The slopes of I(θ) and of the background are the same at these two contact points, and after background subtraction, they correspond to minima of I(θ). In this double logarithmic plot, the line represents a power law behavior of the background. Typically, the background as determined also describes reasonably well the behavior of I(θ) at higher scattering angles θ.

(Fig. 2B). In particular, the four distinct maxima of the inner reflections (interference peaks) (brown in Fig. 2A) appear confluent at their periphery because of the relative rotation in Figure 2B. In the apical scar, the interference peaks (brown in Fig. 2B) show preferred angles of approximately 120°/60°, and they appear unequally intense. In keratoconus, the orientational behavior of the collagen fibrils is best recognized qualitatively by the interference peaks (main maximum (brown) in Fig. 2B), whereas it cannot be obtained easily from the first subsidiary maximum (green in Fig. 2B). Because of the deviation from arrangement in two orthogonal directions, the equatorial contribution (caused by the fibril transform) and the meridional contribution (caused by the axial collagen period) had to be considered separately. This is why a quantitative analysis of the degree of orientation was not attempted for keratoconus.

DISCUSSION

All investigated normal human corneas showed collagen-related intensity profiles characterized by orthogonal fourfold symmetry (Figs. 2A and 3), indicating the preferred orientation of the collagen fibrils along the horizontal (nasal–temporal) and the vertical (inferior–superior) directions. This behavior was found not only in the center of the cornea but also at every other investigated position (nasal, inferior, temporal, superior) at the margin of a central 7 mm zone. In this context, it is noteworthy that the two preferred orientations correspond to the directions of the insertion of the four musculi recti oculi, present in most animals.[20] The preferentially orientated fibrils in the cornea appear as biomechanical elements of a complex optokinetic system that is able to take up the mechanical forces of the musculi recti along the corneal trajectories. Small-angle light-scattering experiments performed on rabbit and bovine corneas also showed such preferred orientation,[13] whereas x-ray scattering experiments in different species only revealed a preferred collagen fibril orientation in the human cornea.[8]

To decide whether the preferred orientation of the collagen fibrils may have some impact for biomechanical consideration, in particular for the development of models of the corneal tissue for refractive surgery, it is a prerequisite to determine quantitatively the amount of fibrils that contribute to the anisotropy. Indeed, current models for the simulation of refractive surgery procedures are based on complex assumptions of the corneal ultrastructure[e.g.,14–16] because detailed information about the orientational arrange-

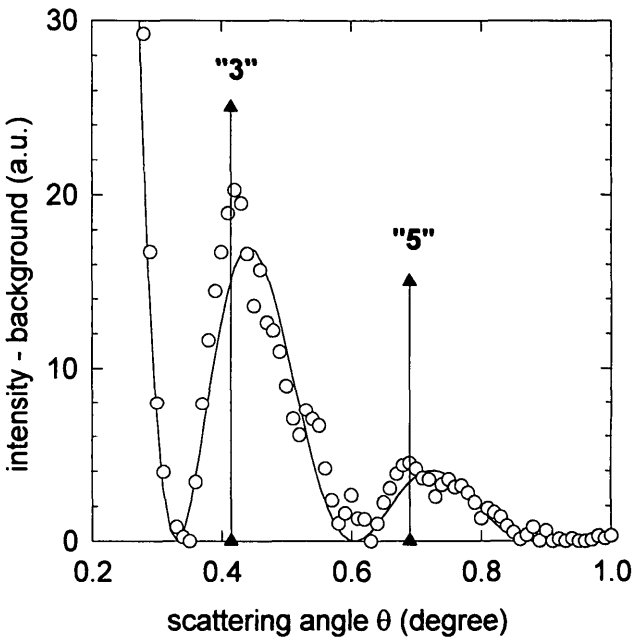

FIGURE 5. I(θ) after subtraction of the background (*circles*). The first subsidiary maximum between 0.35° and 0.6° is clearly visible. The solid curve corresponds to the fibril transform and is proportional to $(J_1(ka)/ka)^2$ (J_1 is the Bessel function of the first kind). The arrows labeled 3 and 5 correspond to the position of the sharp third- and fifth-order peaks of the axial scattering of the collagen fibrils (meridional reflections). Some contribution within the subsidiary maxima caused by this axial fibril scattering is visible.

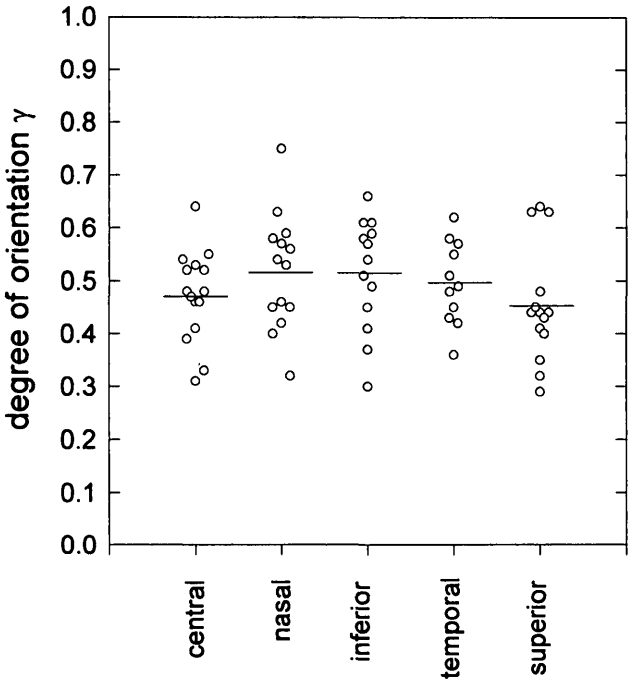

FIGURE 6. Degree of preferred orientation γ for each normal cornea at each of the investigated positions within a central 7 mm zone. The bars show the mean value of each γ-distribution.

ment of the structural elements in the corneal stroma is not available.

Our results show a considerable degree of preferred orientation of γ(mean) = 0.49, which means that the excess of collagen fibrils that represent the preferred orientated portion is 49%. Another value that characterizes the degree of structural anisotropy in the human cornea is the relation between the number of collagen fibrils oriented in the 45° sectors around the horizontal and vertical meridians to the number of collagen fibrils oriented in the oblique sectors between them, which was calculated for γ = 0.49 to be approximately 2:1. In other words, approximately two thirds (66%) of the fibrils are within the horizontal and vertical sectors, whereas only one third (34%) is in the oblique sectors between. The related formula for the probability to find collagen fibrils oriented in a particular direction (see Results) directly permits the implication of the structural data into biomechanical models for the simulation of refractive surgery procedures.

To avoid tractional forces on the specimen caused by mounting, and in accordance with earlier experiments,[4,8,17,21] our measurements were conducted without the application of transcorneal pressure. It has been reported, however, that under conditions in which corneal tension is released, lamellar shape may become wavy.[22] The amplitudes of the waves were shown to be directed perpendicular to the corneal surface, and the periodicity was approximately 10 μm.[22] Because of this geometry, the effect of waviness on the scattering patterns, which were collected with the x-ray beam perpendicular to the corneal surface, were small. One would expect some smearing of the intensity profile $I(\phi)$ and, therefore, a little underestimation of γ.

For unknown reasons, two of the 17 investigated normal corneas (corneas 6 and 9) had a relatively low orientational anisotropy (Fig. 7). Although there was no detectable lesion, the presence of a corneal or systemic disease that may have altered the structure of the corneal stroma cannot be excluded. Alteration of the normal development of the collagen fibril orientation pattern can be induced by the application of

TABLE 1. Degree of Preferred Collagen Fibril Orientation

Cornea Coding	γ				
	Central	*Nasal*	*Inferior*	*Temporal*	*Superior*
1	0.53	0.56	—	0.51	0.45
2	0.52	0.63	0.61	0.57	0.64
3	0.48	0.75	0.66	0.48	0.44
4	0.41	0.45	—	0.45	—
5	0.46	0.57	0.45	0.36	0.63
6	0.31	0.32	—	—	0.35
7	0.52	0.53	0.57	—	—
8	0.47	0.42	0.59	0.58	0.40
9	—	—	—	—	0.29
10	0.55	0.45	0.54	0.62	0.48
11	0.48	—	0.51	0.55	—
12	0.39	0.46	0.49	0.49	0.44
13	0.54	0.59	0.58	—	0.32
14	0.46	0.54	0.30	0.42	0.44
15	0.64	0.58	0.61	—	0.63
16	—	—	0.41	0.43	0.41
17	0.33	0.40	0.37	—	0.43

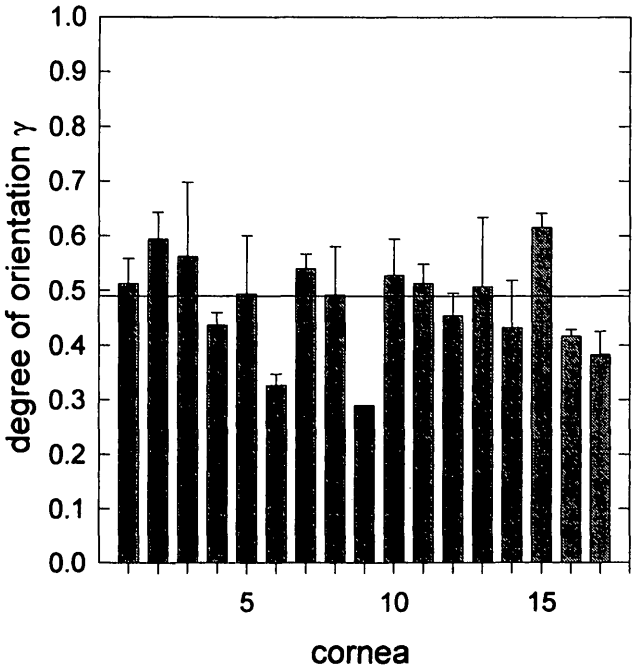

FIGURE 7. Degree of orientation γ averaged over the investigated positions for each of the 17 normal corneas. Bars indicate the standard deviation computed for each cornea. The horizontal line at $\gamma = 0.49$ is a guide for the eye and indicates the mean value for the degree of orientation.

specific drugs, as shown in domestic fowls.[6,7] Despite such experiments, almost no information is available concerning the possible connection between the orientational behavior of collagen fibrils and human corneal disease.

With this in mind, we also investigated four corneas affected by keratoconus by comparing the orientation of the collagen fibrils inside and outside the scarred region. The striking result was that the orientations of the fibrils inside the lesion were altered dramatically in each of the four specimens, whereas outside the diseased region, the orientational arrangement of the fibrils corresponded to that obtained in normal corneas (i.e., orthogonal arrangement). In particular, the angles between the main fibril directions changed to typically 60° and 120° in the apical scar of the keratoconus, and the intensity corresponding to one of the two fibril directions was reduced considerably (see the inner reflections coded in brown [main maximum] in Fig. 2B), indicating an unequal number of collagen fibrils along the main orientations. In other words, the regular orthogonal arrangement of the collagen fibrils, as observed in the normal corneas and in keratoconus outside the lesion, is destroyed within the apical scar of the keratoconus.

Keratoconus is a noninflammatory corneal disease characterized by progressive thinning of the central cornea and accompanied by increased corneal curvature and apical scarring.[23,24] Increased proteolytic activity is probably one of the major events in keratoconus.[25] Proteolytic degradation of collagen fibrils, or even entire lamellae, may be a possible explanation for the abnormal collagen fibril layer arrangement in the apical scar. Some contribution may come from reactive fibrosis in the focal scars within the depth of the stroma.[23] It has been suggested that the adhesiveness of the corneal lamellae may be less than normal in keratoconus.[23] Consequently, one may speculate that the increased degradation process of the extracellular matrix preferentially starts from the lamellar borders. The fact that the orientational behavior of the collagen fibril layers in keratoconus is dramatically altered, whereas changes in other structural parameters of the fibrils are much more subtle,[21] also seems to indicate that ultrastructural disease primarily occurs in interlayer bonding rather than inside the layers.

Key Words

collagen, corneal biomechanics, corneal stroma, keratoconus, refractive surgery

Acknowledgments

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