An interpretation of small-angle light-scattering patterns of human cornea.

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Small-angle light-scattering patterns of human cornea in 1, 1/2, and 1/4 modes were compared to theoretical scattering patterns generated in the computer on the basis of a cornea model. This model is a nonrandom assembly of optically anisotropic rods. The best match of theoretical and experimental patterns yielded the following parameters describing the ultrastructure of human cornea: length of the rod (fiber) = 12 μ; angle between optic and geometric axis of the rod = 80°; most probable orientation of the rod = 150°; the width of the distribution function of rod orientation (σ₁) = 2.5; intrinsic birefringence of the rod = 7 × 10⁻²; form birefringence = 1.3 × 10⁻².

Transparency is one of the most important characteristics of the cornea, and a number of hypotheses have been advanced relating structure and transparency. In our laboratory, we found that in normal bovine cornea low-angle light scattering is due to superstructures of the order of 1 to 20 μ that are symmetrically distributed in the cornea. The scattered light is due both to density and optical anisotropy fluctuation; the latter have two sources, namely, the local intrinsic birefringence and the form birefringence. In order to utilize the information provided in the angular distribution of the scattered light intensity we devised a cornea model. This cornea model is a two-dimensional assembly (idealized lamella) of optically anisotropic rods (collagen fibers) embedded in an isotropic medium (ground substances swollen in water). The rod assemblies (lamellae) have a nonrandom distribution. This means that the lamellae are stacked in a fashion that makes the light passing through them perceive a greater orientation of rods in one specific direction than in the others. This is an interlamellar effect.

With the aid of this model we generated light-scattering patterns in a computer until we matched the experimentally obtained light-scattering patterns. Thus we were able to describe the bovine cornea in terms of six parameters.

The purpose of the present investigation was to obtain small-angle scattering patterns of human cornea and to analyze these in terms of the model proposed for bovine cornea.

Three pairs of human eyes were obtained through the courtesy of Dr. A. Spector of Columbia University, College of Physicians and Surgeons, New York. One patient, a 49-year-old male, died of hypertension at 6:30 P.M., and the excised cornea was used for the scattering experiments less than 24 hours post-mortem. Other corneas came from 75- and 80-year-old male patients, and the excised corneas were used for the scattering experiments at 1:30 P.M., i.e., 19 hours post-mortem. Other corneas came from 75- and 80-year-old male patients, and the excised corneas were used for the scattering experiments less than 24 hours post-mortem.

The excited cornea was placed between a strain-free glass slide and a cover glass. Placing the cornea between flat plates introduced some wrinkles which were, however, avoided by selecting the proper area for exposure to the laser beam. Ten to 12 individual loci on each cornea were exposed to laser beam.

The methods used to obtain the light scattering patterns of human cornea (Fig. 1) are similar to those obtained on bovine cornea. However, normal human cornea scatters about half as much light as normal bovine cornea. This is due to the smaller birefringence exhibited by human cornea as compared to bovine cornea. However, the cornea model developed to elucidate structural features in the bovine cornea can be applied to the human cornea.

In this model we assume that the cornea is made of collagen fibers (rods) whose width is negligible compared to their length, L. The orientation of a rod with respect to the direction of the polarization of the light is given by the angle (Fig. 2). We assume a nonrandom distribution of the rods in different parts of the cornea. The orientation of an assembly of rods is described by a distribution function which is characterized by the maximum of the distribution function σo (i.e., the most probable orientation of an average rod) and by the width of the distribution function, σ. The width of the distribution function can have values from zero (perfect orientation) to 2π (random orientation). This distribution function does not describe a situation where only two perfect orientations of the lamellae are allowed, namely, where each adjacent lamella runs perfectly perpendicular to its neighbors. In addition, the rod is optically anisotropic. The main polarizability axis does not have to coincide with the geometric long axis of the rod, and the angle between the optic and geometric axis, ω, can be non-zero. The nonrandom orientation of an optically anisotropic rod gives rise to an intrinsic birefringence, δ. Furthermore, infinitely thin rods embedded in a medium of different refractive index produce form birefringence, B.

In the development of the theory of the model, we also assumed that all the rods lie perpendicular to the direction of the light; that is, the collagen fibrils run in the plane of the cornea.

Using the equations obtained for the intensity of the scattered light of such a model, one can
Fig. 1. Densitometric scan of light-scattering patterns in the I, and I modes and the best match of computer simulated patterns. $X = 60, \omega = 60^\circ, \alpha_M = 150^\circ, \sigma_s = 2.5, \beta = 7 \times 10^{-5}$, and $B = 1.3 \times 10^{-5}$.

vary continuously, in a computer, the six structural parameters, $\omega, \alpha_M, \sigma_s, \beta, B$, and $X = \pi L/\lambda$ where $\lambda$ is the wavelength of the laser beam. The intensities of the theoretical scattering patterns are obtained as a function of $\Theta$ and $\Omega$ angles of both the I, and I modes. These can be compared to experimental scattering patterns. By selecting the best match between experimental and theoretical patterns, the numerical values of the six structural parameters that yielded the best theoretical patterns are obtained (see Fig. 1).

This is the best comparison between theoretical and experimental scattering patterns originating from the center of the cornea. The theoretical and experimental patterns have common features regarding the angular ($\Theta$ and $\Omega$) dependence of the scattered light both in the I, and I modes. The only discrepancy between the theoretical and experimental scattering patterns is the intensity ratio of $I_1/I$. The theoretical scattering pattern predicts intensity ratios 2 to 2.5 times as high as found experimentally, especially at wider angles. No amount of manipulation of the six parameters of our model could decrease this discrepancy.

Fig. 2. Model of an optically anisotropic rod. Z, Direction of the polarization of the laser beam (normal to the optic axis); $S_i$, incident beam; $S_s$, scattered beam; $\Theta$, scattering angle; $\Omega$, azimuthal angle; $\alpha$, orientation of the rod; $\omega$, angle between geometric and optic axis of the rod; $L$, length of rod.
However, such disagreements are not unusual when attempts are made to reconcile experimental data with model systems.

If the two-dimensional nonrandom assembly of anisotropic rods is considered to be a suitable model for human cornea, the following observations can be made regarding the numerical values of the parameters in Fig. 1.

The value $\lambda = 60$ corresponds to a length of the scattering rod of $12 \mu$. This is about half as much as we obtained for the bovine cornea (26 $\mu$). The question can be asked whether such a unit corresponds to any structural units observable under the light or electron microscope. Although the generally accepted view is that in the stroma there are only two levels of organization, namely, fibrils and lamellae, Kikkawa claimed that structural units larger than fibrils do exist in the cornea.

Diffraction phenomena and the appearance of the stroma under polarizing microscope promoted the postulation of units in the order of microns. Maurice is of the opinion that these dimensions may correspond to the fine corrugation of the lamellae caused by the waviness of fibrils. Thus the dimension of $12 \mu$ as the length of the scattering rod may correspond to aggregates of fibrils forming fibers that are visible under the light microscope.

In the human cornea the collagen fibers have an optic axis that is $60^\circ$ from that of the geometric axis. Similar values were found for bovine cornea and also for collagen films. We are of the opinion that this $\omega = 60^\circ$ represents the helical winding of the tropocollagen aggregates within the fibrils.

The consequence of $\omega = 60$ is that the maximum of the distribution function of rod orientation will occur at $150^\circ$. In order to minimize the effect of birefringence upon the light scattering pattern, we aligned the sample in the $1+0$ mode so that its optic axis was normal to the polarization of the laser beam. Hence $\omega = 90^\circ = \alpha_0$ is the consequence of our experimental procedure.

The width of the distribution function of the rod orientation was $\sigma = 2.5$, which means that the net effect of stacking the lamellae is the production of greater randomness in human cornea than in bovine cornea. The consequence of this is that the intrinsic birefringence of human cornea is less than that of bovine cornea. This was also demonstrated by the value obtained for $\delta = 7 \times 10^{-3}$ for human versus $2 \times 10^{-3}$ for bovine cornea.

The value obtained for form birefringence is also low—$B = 1.3 \times 10^{-3}$ for human cornea against $4 \times 10^{-3}$ for bovine cornea.

Form birefringence was obtained in a separate experiment when human cornea was immersed in liquids with different refractive indexes. The proportion of form to total birefringence was found to be similar to that reported by Maurice. Only two factors can affect the form birefringence: the refractive-index difference between the fibril and the surrounding ground substances and the volume fraction of the fibrils. Since the refractive-index difference enters into the Wiener equation as a squared term, it is more likely that slight differences in the refractive indexes of the surrounding media are responsible for the difference in form birefringence between human and bovine cornea. The fact that both intrinsic and the form birefringence are much smaller in human cornea than in bovine cornea is confirmed by the comparative mapping of the total birefringence isochores in these two species.

The application of a nonrandom assembly of anisotropic rod model to the light-scattering patterns of human cornea demonstrated that the parameters obtained from light scattering can describe interesting features of the ultrastructure of the cornea.

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REFERENCES

Small-angle light-scattering patterns of corneas of different species. FREDERICK A. BETTELHEIM AND ROBERT MAGRILL.

Light-scattering patterns of corneas of different species have been obtained. The different small-angle light-scattering (SALS) patterns were classified into four groups on the basis of the angular dependence of the intensity of light scattered in the I and I modes. At present, only two types of patterns can be explained on the basis of theoretical models. The need to develop a general model that can account for all four types of SALS patterns is discussed.

Normal corneas scatter light appreciably only at small angles (0° to 4°). Scattering occurs both in the I and I modes, that is, when polarizer and analyzer are aligned perpendicular or parallel. This means that the scattering is due both to density and optical anisotropy fluctuations. Thus, in principle, scattering patterns can be used to analyze the ultrastructure of cornea. Electron microscopic investigations seem to imply similar alignment of collagen fibrils in the lamellae and similar packing of the lamellae in the cornea of different species. On the other hand, corneas of different species had distinct and different SALS patterns that would imply distinct and different ultrastructures. Bovine cornea showed a light-scattering pattern that can be accounted for by a model of nonrandom assembly of anisotropic rods. Rabbit cornea implied a sheaflike morphology. Rat cornea yielded a SALS pattern that could not be accounted for by either of these models. Clearly there is a need to develop a model that can account for all types of corneal light-scattering patterns. The first step in this direction requires the investigation of the number of and nature of different light-scattering patterns yielded by corneas of different species.

Bovine (Bos taurus) and lamb (Ovis aries) eyes were obtained from slaughterhouses less than 12 hours post-mortem. Sea bass (Micropterus salmoides), sea trout (Salmo trutta), and bluefish (Pomatomus saltatrix) were obtained from fishing vessels less than 10 hours post-mortem while the fish were kept in seawater. Carp (Cyprinus carpio), chicken (Gallus gallus), turkey (Meleagris gallopavo), duck (Anas platyrhynchos domesticus), pigeon (Columba livia), and frog (Rana temporaria) were sacrificed just before enucleation. Human corneas (Homo sapiens) were obtained less than 24 hours post-mortem.

Small-angle light-scattering (SALS) patterns were obtained as described earlier. The SALS patterns of corneas of different species can be classified on the basis of the variation of the intensities in the I and I modes with scattering angle θ and azimuthal angle Ω. Group I (Fig. 1, a and b) is characterized by a four-lobe scattering pattern in the I mode in which one set of the lobes is more prominent than the other set normal to it. The corresponding I pattern does not have this four-lobe pattern but have a geometric anisotropy along the direction of one set of the lobes in the I pattern. Both I and I patterns have only one intensity maximum. The patterns in Fig. 1, a and b, come from a bovine cornea, and the distribution of such patterns was studied extensively. Human corneas provide in essence the same SALS patterns as bovine corneas.

SALS patterns of Group II (Fig. 1, c to h) are characterized by a cloverleaf arrangement in the I mode. This cloverleaf pattern has five intensity maxima as compared to the one maximum of the first group. On the basis of more detailed structure, group II can be divided into three subgroups. Group IIa (Fig. 1, c and d) has an I pattern in which the cloverleaves are in a 0° to 90° arrangement. The corresponding I pattern is nondescript. Chicken, turkey, duck, bluefish, sea bass, and rat corneas yield such patterns.

Group IIb differs from IIa only in the I mode. The four lobes of the cloverleaf are not in a 0° to 90° arrangement but at 0° to less than 90° arrangement (Fig. 1, e and f). Otherwise the I of this group is just as nondescript as it was in Group IIa. Frog and carp corneas provide SALS patterns belonging to this group.

Group IIc has I patterns in which the four lobes of the cloverleaf are at 0° to less than 90° arrangement, just as in Group IIb, but the I pattern is anisotropic (Fig. 1, g and h). The I pattern has a direction along one set of the cloverleaf lobes of the I pattern. In this sense there is a similarity between Group I and Group IIc, but the basic difference, that is, five maxima vs. one maximum in the I patterns, separates them. Sea trout and rabbit corneas yielded such patterns.

The less than 0° to 90° cloverleaf arrangement requires a special comment. It has been shown that deformation of the rod or spherulite structure due to elongation can cause such an effect. It is also known that cornea under different tensions gives rise to different SALS patterns. In our cases the excised corneas were under no tension, pos-