Interlamellar Cohesive Strength in the Vertical Meridian of Human Eye Bank Corneas

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**Purpose.** Previously, human corneal stromal interlamellar cohesive strength in the horizontal meridian was shown to be twice as strong peripherally as centrally (~2.90 × 10^-1 versus 1.40 × 10^-1 N/mm). In the current study, stromal samples excised from the vertical meridian were studied to determine if meridional differences also exist. Precise knowledge about corneal stromal structure is warranted, because anisotropy can influence postoperative healing strength and corneal shape.

**Methods.** Limbus-to-limbus stromal strips with a constant 2.2-mm width were obtained from the vertical meridians of 52 eyebank corneas (8 single, 22 pairs). Cohesive strength was recorded as samples were split at a constant 1.6 mm/s at 50% stromal depth.

**Results.** The mean cohesive strength in the inferior periphery was found to be only two thirds the strength observed in the nasal or temporal periphery (1.96 × 10^-1 N/mm versus ~2.94 × 10^-1 N/mm @ 5 mm). The mean cohesive strength in the inferior periphery was also significantly less than the strength of the superior periphery (1.85 × 10^-1 N/mm ± 8.83 × 10^-3 SEM versus 2.34 × 10^-1 ± 1.37 × 10^-2 @ 4 mm from the central cornea; P = 0.0027). Unlike the samples from the horizontal meridian, which could be described by a profile symmetric about the central cornea, force profiles for the vertical data could be characterized with distinct, classifiable patterns that were generally asymmetric. Fellow corneas from a single donor tended to have strength profiles belonging to the same class (P = 0.035; chi-square), although some paired eyes exhibited profiles from distinctly different classes.

**Conclusions.** These data strongly support the concept of an anisotropic collagen macrostructure that is more complex than previously believed. This inherent structural anisotropy may become a significant determinant of corneal shape during ectatic disease and some forms of keratorefractive surgery. Invest Ophthalmol Vis Sci. 1993;34:2962-2969.

The biomechanics of keratorefractive surgery and stromal wound healing strength have brought about a deeper appreciation for the significance of corneal structural organization and material properties as determinants of surface topography and stromal transparency.1-9 Theoretically, biomechanical modeling of the cornea can be made more robust by replacing current assumptions about stromal homogeneity and isotropy with empirical data for corneal structure.3,6-7 However, the current state of finite-element computer modeling limits the ability to create and manipulate a truly realistic representation of the cornea. Nevertheless, new insight into corneal structure may inspire innovative approaches to keratorefractive surgery, establish parameters to be incorporated into refined biomechanical models, and suggest improvements in the control of wound healing. Perhaps it may even become possible to fine-tune a surgical procedure for an individual eye, based on specific structural features and biomechanical behavior. Currently, only very general information about the structural organization and material properties of the cornea is known. Some of this information has become a subject of debate in recent years, having been based on technically inade-
The stromal splitting technique was first reported by Maurice with rabbit corneas,14 and was subsequently applied to human tissue to determine the anteriorposterior binding characteristics of the cornea along the horizontal meridian.15,16 Human stroma was found to be weakest centrally (1.39 × 10−1 N/mm ± 4.90 × 10−3 SEM) and strongest peripherally (∼3.10 × 10−1 ± 3.63 × 10−2 SEM), in contrast to the rabbit stroma, which had a nearly constant strength across the entire cornea when measured by the same method (8.92 × 10−2 N/mm).16 These findings corroborate the differences in the basic nature of the normal, intact tissues; excised rabbit corneas are firm and more resilient to manipulation. The anatomic basis for this disparity appears to involve a fundamental species difference in the structural organization of the tissue. This issue has not been fully explored, and extends beyond the arguments about the significance of Bowman’s layer to tissue rigidity.

In humans, cohesive strength was correlated with the histologic appearance of depth-varying collagen lamellae that crossed the interlamellar splitting plane, and increased in number toward the periphery.15,16 Furthermore, there have been reports of nonlamellar, depth-varying collagen bundles with bifurcations at various depths that appear to tie together the anterior stroma and Bowman’s layer to more posterior levels of the stroma.15,16,19 Precise information about the distribution and mechanical significance of these and other structural features remains unknown.

During testing of human eye bank tissue along the horizontal meridian,19 cursory testing of the inferior and superior remnants indicated a cohesive strength disparity, with the superior remnant always stronger than the inferior remnant. Because this may be a highly significant feature with respect to the distribution of stromal mechanical stress, and because it suggests a meridionally dependent organizational difference, a complete study of vertical meridian cohesive strength was performed.

METHODS

Fifty-two adult human donor eyes obtained from local eye banks (8 single and 22 pairs) were stored in moist chambers at 4°C until use within 72 hours. Donors ranged in age from 38 to 92 years with a mean age of 69.5 years. Distribution of the donors according to sex was 60% male and 40% female. No known corneal dystrophy or prior surgical condition was present. Corneal samples were prepared according to a method described previously,15 however, the strip samples were obtained from the vertical and not the horizontal meridian. In brief, 2.2-mm wide corneal stromal strips were excised along the vertical meridian passing through the central cornea, with a 3–4-mm scleral margin remaining intact. While viewing under a surgical microscope at 20× power, either the superior or inferior end of the strip was carefully cleaved with a scalpel blade at a 50% depth through the sclera and approximately 2 mm into the stroma to initiate a splitting plane. As an aid in determining when the end of the stromal strip was reached during the splitting experiment, the uncleaved scleral end of the strip was removed at the limbus. The posterior portion of the cleaved end was attached to an immobile base hook, while the anterior portion was attached to a vertically translating, isometric force transducer that travelled at a constant rate of 1.6 mm/s. The force generated during the splitting of the strip was recorded on a strip-chart for future analysis. When the central cornea was split (determined visually by the location of an ink mark at the midpoint of the strip), the event was indicated on the chart. Excising, mounting, and testing each sample took approximately 3 minutes. Each chart recording was later digitized into a computer.
RESULTS

The mean cohesive strength profile for the vertical meridian of all corneas in this study is shown in Figure 1. For comparison, the mean data from the horizontal meridian from the previous study are also shown. The most distinguishing characteristic of the vertical meridian data was the pronounced drop in cohesive strength in the inferior periphery compared to data from the other principal semimeridians. The cohesive strength in the inferior periphery was significantly less than the strength of the superior periphery at and beyond 3 mm arc length at the \( P = 0.05 \) level (Table 1). Likewise, the nasal and temporal mean strength values at 4 mm and beyond were significantly different from values in the inferior semimeridian; the inferior periphery at 5 mm arc length had approximately two thirds the cohesive strength of the other primary semimeridians.

Centrally, the vertical profile had a "dip" similar to that found in the horizontal profile. The central vertical value was greater than the horizontal value (1.65 \( \times 10^{-1} \) N/mm \( \pm 8.83 \times 10^{-3} \) SEM versus 1.39 \( \times 10^{-1} \) \( \pm 4.90 \times 10^{-3} \) SEM), but they were not significantly different (\( P = 0.11 \)). Because the strip samples were orthogonally oriented and crossed at the central cornea, and because the strength of the samples reflected the integrated value across their width, it was expected that the central value of either the vertical or horizontal strip would have a value similar to the \( \pm 1 \) mm value of the orthogonal strip. For the human corneas, the horizontal strength value at 1 mm on either side of the central cornea was found to be similar in magnitude to that of the central data point of the vertical meridian. That the central horizontal value was less than the \( \pm 1 \) mm vertical sample values may be tentatively explained by a region with reduced lamellar interweaving that is longer vertically than wide horizontally. It was found that the central midstroma had minimal lamellar interweaving compared to paracentral and peripheral regions.\(^{15,16}\)

Figure 2 gives examples of corneal cohesive strength profiles from fellow eyes in each category of the classification system. This classification system was based on a qualitative assessment of the relative shape of all profiles; the corneal strength profiles appeared to gradually vary from one class to the next, with the exception of class 1, which was based on a unique pair of corneas. All strength profiles were randomly screened several times, and consistently grouped into the same categories. Table 2 gives the number and percentages of corneas in each class. For paired corneas, 16 of 44 (36.4%) were within the same class as their fellow corneas, and 30 of 44 (68.2%) were within one class. Using a chi-square nonparametric cross-correlation test between right and left fellow corneas, the probability of donor pairs having strength profiles of the same class was significant (\( P = 0.035 \)).

Class 1 was unique with only one pair of corneas demonstrating reduced superior strength with a rise in strength in the inferior periphery where a pronounced
Corneal Cohesive Strength

![Graphs showing cohesive strength profiles for different classes.](image)

**FIGURE 2.** Cohesive strength data from fellow corneas. Classification was determined by relative profile shape. Note the general concordance between the fellow corneas both in terms of shape and strength magnitude. Age and sex of the donors are indicated in the corners of each plot.

A dip in strength was observed. Class 2 corneas exhibited a constantly diminishing strength profile from the superior to the inferior periphery. Class 3 corneas had either a gradual (3a) or sharp (3b) drop in cohesive strength inferiorly. These corneas were grouped within a single class because the profiles strongly suggest a continuum of change between the two subclasses. Class 4 corneas had relatively flat or slightly undulating strength profiles across most of the central and inferior paracentral cornea. Finally, class 5 had a minimum strength value centrally with an increase in strength superiorly and a nonsymmetrical, smaller rise in strength inferiorly. No trends specific to donor age, sex, or postmortem time of testing were noted.

In addition to the distinct patterns by which these corneas could be classified, there was also a noticeable difference in the magnitude of the strength profile fluctuations among the classes. For example, class 5 profiles had relatively large, spiky features, whereas those for class 3 tended to have lower magnitude fluctuations, particularly in the inferior periphery where cohesive strength was weakest. The peak to peak fluctuation magnitude, standard deviation of the mean fluctuation, and the coefficient of variation were examined for regions in the inferior and superior periphery in all seven samples of class 3a (Table 3). The fluctuations in cohesive strength in the inferiorly depressed region were significantly different from the superior region at the $P = 0.01$ level for all measures.

The strength profiles for the vertical meridian were initially split in either the superior to inferior or inferior to superior direction (Figure 3). There was no distinct difference noted between the overall shape of the profiles generated by the two splitting directions; inferior to superior splitting also exhibited profiles with reduced inferior cohesive strength. However, the process of initiating a cleaving plane caused the loss of data near the limbus, so samples later in the study were split from superior to inferior to preserve the inferior data.

Several corneas were tested using side-by-side strip samples (Figure 4). One sample was centered on the vertical meridian, and a second sample obtained from either the nasal or temporal side. In all cases, the two samples were similar in basic profile, but showed slight differences, especially in the inferior region. These profiles suggest that the region of reduced strength in the inferior cornea extends laterally from the vertical meridian and may not necessarily be centered with its lowest cohesive strength precisely on the vertical meridian. Perhaps some of the profile variations found among the classes may be attributed to a region of cohesive weakness offset from the vertical

### TABLE 2. Cohesive Strength Profile Classification Summary Statistics

<table>
<thead>
<tr>
<th>Class</th>
<th><strong>Paired and Single Eyes</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>All n (%)</strong></td>
<td><strong>OD n (%)</strong></td>
<td><strong>OS n (%)</strong></td>
</tr>
<tr>
<td>1</td>
<td>2 (3.8)</td>
<td>1 (3.6)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td>2</td>
<td>9 (17.3)</td>
<td>5 (17.9)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>3a</td>
<td>7 (13.5)</td>
<td>3 (10.7)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>3b</td>
<td>7 (13.5)</td>
<td>3 (10.7)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>4</td>
<td>15 (28.8)</td>
<td>8 (28.6)</td>
<td>7 (29.2)</td>
</tr>
<tr>
<td>5</td>
<td>12 (23.1)</td>
<td>8 (28.6)</td>
<td>4 (16.7)</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>28</td>
<td>24</td>
</tr>
</tbody>
</table>

Superior = minus values  Inferior = positive values
meridian, so that the sample passes across a portion of the region with marginal weakness.

DISCUSSION

It appears unlikely that the results of this study relate to the depth of the splitting plane. Ultrasonic pachymetry and digital micrometry were used to confirm splitting depth, which was found to vary from a 50% depth by no more than 10%. It seems doubtful that the specific classifiable differences in cohesive strength that have been observed could occur within such a narrow range of depth. The strong similarity of strength profiles observed between fellow corneas (Figure 2) also would be unexpected if stromal organization were random or highly dependent on depth of the splitting plane. Asymmetrical profiles of the type found in the current study were not noted in the previous study of the horizontal meridian using a very similar protocol, however, a correspondence in the relative profile flatness or steepness was observed between fellow corneas. 15, 16

The mean strength profile obtained in the current study has a centrally located region of cohesive strength reduction and a secondary region of reduced strength inferiorly (Figure 1). Among individual corneas, a region of reduced cohesive strength varies in magnitude, extent, and location; however, some paired corneas exhibit remarkably similar profiles (Figure 2 and Table 2). In class 4 corneas, a region of diminished strength extends well beyond the central cornea, resulting in profiles that appear generally flat, whereas in other corneas, such as those of class 5, the central region of reduced cohesive strength is well delineated and concomitant with the geometric center of the cornea.

For the average cornea, the inferior portion of the profile exhibited significantly reduced strength based

<table>
<thead>
<tr>
<th>Superior Semimeridian</th>
<th>Inferior Semimeridian</th>
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<tbody>
<tr>
<td>Cornea</td>
<td>P-P (N/mm) (x10^-3)</td>
</tr>
<tr>
<td>1</td>
<td>30.6</td>
</tr>
<tr>
<td>2</td>
<td>28.6</td>
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<tr>
<td>3</td>
<td>31.4</td>
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<td>4</td>
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<tr>
<td>5</td>
<td>47.0</td>
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<tr>
<td>6</td>
<td>121.2</td>
</tr>
<tr>
<td>7</td>
<td>86.1</td>
</tr>
</tbody>
</table>

Paired Student’s t-test probability measures for superior versus inferior differences: P-P, P = 0.01; SD, P = 0.004; CV, P = 0.005. P-P = peak to peak; SD = standard deviation; CV = coefficient of variation.

FIGURE 3. Cohesive strength profile from a strip split in the inferior to superior direction. Note that the inferior strength does not start with a high value; strength profile shapes were not dependent on direction of the splitting.

FIGURE 4. Cohesive strength profiles from adjacent strip samples from a single cornea. Note the basic similarity between the profile shapes, with a strength difference in the inferior paracentral and peripheral region.
Corneal Cohesive Strength

on comparisons of mean values (Figure 1 and Table 1). It is unknown whether the structural features, or perhaps more correctly, the lack of structural features that cause this strength reduction are necessarily centered with respect to the excision axis of the strip samples. Therefore, the variations in profile strength may be due to both variations in the magnitude of a structural deficit (e.g., density of interweaving collagen lamellae) and to the lateral spatial relationship of the region of strength reduction to the vertical axis. A nasal or temporal offset of a graded region of reduced strength could alter the appearance of the strength profile in a manner similar to a pure magnitude change for a vertically centered region. Side-by-side strip samples indicate that the region of reduced strength does indeed extend beyond the region of the central sample, and the magnitude of reduced strength is not necessarily at a minimum on the vertical meridian (Fig. 4). Thus, the various classes described in this study may actually reflect a continuum among all corneas, whereby cohesive strength in the stroma varies with a spatially mediated magnitude component.

The magnitude of the strength function at any given point along the profile is the integrated strength across the width of the strip at a specific distance relative to the central cornea. Thus, it may reflect the number, dimensions, and/or tensile strength of stromal collagen fibrils interweaving in that portion of the strip sample, with a possible contribution by the material properties of the ground substance. \(^1\) Cohesive strength has been shown to be associated with the relative number of torn collagen bundles crossing the interlamellar splitting plane. \(^15\) Recent histologic evidence indicates that there is interlamellar sharing of fibrils through bifurcation, fusion between adjacent, parallel sheets of fibrils, and even single fibril strands of collagen being shared between adjacent lamellae. \(^15\) Such evidence supports the circumstantial observations of fibril strands occasionally seen traversing the artificial interlamellar spaces in histologic thick sections of swollen rabbit stroma. \(^16\) More study is needed to determine the spatial extent of regions of cohesive strength deficit, both laterally and with depth in the stroma. A more precise method is also needed to equate cohesive strength to specific structural features such as the magnitude of interweaving or lamellar bifurcations within a given plane.

The localized fluctuations in strength seen as spikes in the profiles may be related to the tensile stretching and abrupt tearing of specific collagen structures crossing the splitting plane, such as depth-varying bundles of collagen. \(^16\) The change in relative magnitude of these fluctuations, particularly the reduction observed in the inferior periphery of class 3 corneas cannot be explained at this time, but may be caused by a reduction in numbers of depth varying bundles, material property differences at the fibrillar level, reduced dimensional aspects of the collagen bundles, or an unknown organizational change among the lamellae.

It is not surprising to confirm that the human corneal stroma has an unequal cohesive strength distribution from the central to peripheral cornea based on data from the earlier study, \(^15\) however, it is interesting that this strength distribution is not radially symmetrical about the central cornea. Data from rabbit corneas for the horizontal meridian did not indicate a centrally dependent strength profile, being relatively flat on average. \(^16\) Although there have been a number of studies dealing with preferred orientation of collagen fibrils both centrally and peripherally no mention has been made of meridionally dependent effects that could play a role in cohesive strength. \(^20\) It is unlikely, however, that histologic data about orientation alone could be correlated with cohesive strength differences. Maurice and Monroe have described a well-reasoned account for possible mechanisms of cohesive strength. \(^14\) Histologic evidence for these features, particularly with respect to stromal location and species, should be pursued.

It is intriguing that topographic deformations associated with corneal keratoconus and pellucid marginal degeneration appear to share similar regional specificity with the inherent cohesive strength weakness found in this study. \(^25\) For example, class 3 profiles suggest an inferior limbal weakness localized to the area typically associated with pellucid marginal degeneration, class 4 suggests a broad region of strength deficit as might be associated with an oval cone, whereas class 5 has a central weakness befitting the round or nipple type of cone. To date, there has been no adequate explanation for the form variations seen in keratoconus or the tendency for inferiorly localized cones. \(^25\) Although there appears to be a strong association between connective tissue disease and keratoconus, the cause of the disease is still a mystery; eye rubbing, atopy, contact lens wear, and various hereditary disorders have all been suspected of playing a role in the disease. \(^26\) Although the correlation between cohesive strength and ectatic deformation is circumstantial, there should be further study of the influence of a normally occurring, structural anisotropy in the development and progression of specific forms of topography in ectatic disease.

The ultimate determinants for corneal shape are the structural elements of the tissue itself and the magnitude and direction of forces distributed within the load-bearing structures. Such factors are the basis for some keratorefractive procedures such as thermoker-
atoplasty, which alters the organization of intrastromal collagen and establishes new load bearing collagen cross links when reshaping the cornea. If specific midstromal load-bearing structures are either lacking or reduced in strength as indicated in this study, then these corneas may be more predisposed to form specific types of abnormal topography when a disease such as keratoconus occurs. Because keratoconus appears to first develop as a degeneration of anterior corneal structures that progresses posteriorly into the stroma, the biomechanical response of the middle and posterior stroma may become relatively more significant to corneal shape as the disease progresses over time. Localized thinning of the tissue may be related to the increased susceptibility to shearing forces in regions with a cohesive strength deficit. The entamor- phism of the keratoconic pattern observed in some fellow eyes may be based not on the disease per se, but on a similar pattern of anisotropically distributed load bearing structures in the stroma of the fellow eye. The correlation of cohesive strength profile classification between fellow eyes may reflect a similar correlation for keratoconic entamorphism.

In conclusion, this study has shown that the mean interlamellar cohesive strength is diminished in the inferior peripheral portion of the cornea compared to values obtained from the other principal meridians. The mean cohesive strength in the central cornea was shown to be less than that of the superior periphery, and less than the previously published results for the nasal and temporal periphery. The biomechanical significance of this deficit is probably minor if not irrelevant in the average, intact cornea under physiologically normal tensile loading, but may become significant in corneas with specific forms of degenerative disease or trauma that induces shearing strains between the lamellar sheets of the stroma. Further study of the structural organization of the cornea is needed, including computer simulations of depth-varying structures on stress distribution and topography. The effects of an inherent, localized cohesive strength deficit in mediating the course of ectatic deformation should be considered as a potentially important mechanism. The implications of the results of this study also may be of practical importance to refractive surgery. Anisotropy in the stroma may become an important topographic factor in deep photorefractive keratoplasty ablations, or may gradually influence the results of refractive procedures during the lifetime of the patient.

Key Words

cornea, corneal biomechanics, corneal stroma, keratoconus, corneal cohesive strength

References

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Corneal Cohesive Strength


