Interlamellar Adhesive Strength in Human Eyebank Corneas

Michael K. Smolek and Bernard E. McCarey

The interlamellar biomechanical properties of stromal collagen are relatively unknown, yet may be highly significant with respect to wound healing and the efficacy of certain keratorefractive surgical procedures. Interlamellar adhesive strength was measured as the tearing force required to separate corneal lamellae at a 50% stromal depth in 16 human eyebank corneas. The mean value for the central cornea was found to be 14.2 (±0.5 SEM) g-wt/mm of tissue width. Histology showed a smooth separation between the lamellae along the tearing plane in the central cornea. We believe that the adhesive strength measured in the central cornea may be primarily the force needed to break interlamellar proteoglycan bonds between collagen lamellae, because no torn lamellae were found in this region. The mean adhesive strength and the SEM increased toward the periphery in a symmetrical fashion. The mean adhesive strength in the far periphery was 31.6 (±3.7 SEM) g-wt/mm at 5 mm nasally, and 28.4 (±3.2 SEM) g-wt/mm at 5 mm temporally, and was approximately twice the mean central value. The rising value of the mean adhesive strength with increasing distance from the central cornea was believed to be due to a more highly disorganized collagen network in which greater numbers of lamellae passed obliquely in depth through the tearing plane. These lamellae would contribute their tensile strength to the adhesive strength measurement along the tearing plane. Histology from the peripheral cornea confirmed the existence of depth-varying collagen lamellae and the torn ends of lamellae that passed across the tearing plane.


A variety of methods have been used to investigate the biomechanical properties of the eye. However, little data has been gathered on the adhesive strength between lamellar planes that lie parallel to the corneal surface. Evaluation of interlamellar adhesive strength and its location specificity is important for understanding stromal wound healing characteristics for various lamellar keratoplasty procedures, and would provide fundamental information about the biomechanical relationships between collagen lamellae and proteoglycan complexes (ie, glycosaminoglycan side chains attached to a protein core).

The biomechanical strength of interlamellar adhesion in the rabbit cornea has been investigated by Maurice and Monroe. They measured the tearing force required to separate the rabbit stroma along an interlamellar plane, and found it to be approximately 90 g-wt/mm of sample width. We found that this value to be unreasonably large and so needed to be verified. In a recent paper by Maurice and Monroe, it was reported that the value for the tearing force was actually closer to 10 g-wt/mm. They also reported that the tearing strength was essentially constant across the entire cornea. Some corneas had a rise in tension near the limbus. We found this result to be noteworthy because one would expect the tearing force to increase in the corneal periphery, where a greater degree of collagen interweaving has been reported.

The purposes of this study were to measure the mean interlamellar adhesive strength in human eyebank corneal stroma; to observe any change in adhesive strength with peripheral locations; and to ascertain what morphologic features contribute to adhesive strength.

Materials and Methods

Sixteen whole-globe human donor eyes enucleated 1–3 hr postmortem were obtained from the Georgia Lions Eye Bank (Atlanta, Georgia). The eyes appeared normal, and no known corneal dystrophy or prior surgical condition was present. These eyes were maintained under refrigeration in a moist chamber until the time of the experiment (<120 hr postmor-
Loose conjunctival tissue around the limbus was removed with corneal scissors, and the superior and nasal directions were marked with ink on the anterior sclera. An incision was made into the sclera approximately 4 mm from the limbus with a no. 11 scalpel blade. Corneal scissors were used to cut the cornea from the globe while maintaining a 4-mm scleral rim. The corneal segment was handled along the scleral rim with fine-tooth forceps, and the lens, ciliary body, and iris were removed from the detached corneal segment with forceps.

The corneal segment was placed anterior side up on a convex polyethylene surface that closely matched the curvature of the posterior cornea. A strip-cutting knife constructed of two surgical grade razor blades separated by a width of 1.8 mm was used to cut a limbus-to-limbus sample along the horizontal meridian of the cornea. To produce a sample of uniform width, the knife was rotated across the plastic surface as the incisions were made (Fig. 1). The midpoint of the length of the sample was marked with ink and considered to be the geometric center of the cornea (i.e., the zero point of corneal arc length).

A 30-gauge hypodermic needle was inserted perpendicular to the nasal limbus, through the corneal strip, and into the plastic surface to stabilize the sample. A no. 11 scalpel blade was inserted at 50% depth into the stroma, 1–2 mm from the nasal limbus. The blade was gently rocked into the tissue parallel to the surface until the tip emerged on the opposite side of the sample, and the cutting edge was against the hypodermic needle. The needle was removed, and the scalpel blade was passed further into the sample so that the limbal and scleral tissue was cleaved into two separate layers.

A 5-mm-long hook attached to a chain was inserted into the hole produced by the hypodermic needle on the anterior layer of the sample. The free end of the posterior layer was attached in a similar fashion onto a hook rigidly mounted to the base plate of a vertical lead screw strip pulling device (Fig. 2). Figure 3 shows how the tissue was torn along the length of the sample. The use of hooks was found to be preferable over the inconsistent adhesive quality of cyanoacrylate glue, and less tissue manipulation was required compared to the use of clamping grips. The hooks did not pull through the ends of the strips because of the highly interwoven nature of the attached scleral rim. Distortion within the tissue during tearing did not extend beyond 1–2 mm from the hook.

The end of the chain was fastened to a Gould Metrigram isometric force transducer attached to the traveling base of the lead screw. A Gould RS 3200 strip-chart recorder displayed the output from the force transducer. As the tearing plane passed the marked central cornea, the event was indicated on the strip-chart recording. Instrument calibration was performed before and after each experiment by the application of a known force to the transducer. The tissue sample was loaded at a constant speed of 1.6 mm/sec. The time required to isolate, mount, and test a sample was less than 3 min.
Results

Tearing Force Measurements

Strip chart output was reduced by determining the tearing force at intervals equivalent to 0.16 mm corneal arc length, and replotted with fellow-eye data as shown in the examples in Figures 4 and 5. Corneal arc length was the distance in millimeters from the corneal geometric center toward the nasal (negative) and temporal (positive) directions. Adhesive strength was the force in gram-weight per millimeter of sample width required to tear apart the anterior and posterior layers of the sample. Second order regression lines for each sample were plotted as solid (left eye) and dashed (right eye) lines. This least-squares fit of the regression line to the data was made in the form of the second-order polynomial equation:

\[ Y = a_0 + a_1X + a_2X^2 \]

where \( a_0 \), \( a_1 \), and \( a_2 \) are polynomial regression coefficients.
Table 1. Summary statistics for human interlamellar adhesive strength

<table>
<thead>
<tr>
<th></th>
<th>Corneal arc length (mm)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nasal</td>
<td>Temporal</td>
</tr>
<tr>
<td>Mean</td>
<td>31.6</td>
<td>30.1</td>
</tr>
<tr>
<td>Maximum</td>
<td>47.3</td>
<td>49.8</td>
</tr>
<tr>
<td>Minimum</td>
<td>23.0</td>
<td>17.0</td>
</tr>
<tr>
<td>SD</td>
<td>9.0</td>
<td>10.2</td>
</tr>
<tr>
<td>SEM</td>
<td>3.7</td>
<td>2.9</td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>12</td>
</tr>
</tbody>
</table>

The table lists the summary statistics for the data at 1-mm arc length intervals for all corneas. The limits of the nasal and temporal values differed for each cornea (and thus the n values decreased) because of differences in the prepared length of a sample and in the original dimensions of each cornea.

Fluctuation in the tearing force with respect to corneal arc length can be observed in the data plots of individual eyes (Figs. 4, 5). We are uncertain as to the precise structural origin of these variations; however, they may be related to the organization of the lamellae in the stroma. These fluctuations directly demonstrate the existence of nonhomogeneous material properties of the stroma.

Figure 6 is a plot of the mean adhesive strength in gram-weight per millimeter (±SEM) versus the corneal arc length at 1-mm intervals for all 16 corneas. This plot shows that the peripheral values near ±5 mm differed from the central value by a factor of 2 in relative magnitude, and that adhesive strength is functionally symmetric about the central cornea in the horizontal meridian. Note that the SEM increases toward the more peripheral locations. Although a portion of this variance is due to biologic variability among the eyes (eg, note the difference in curvature between the plots of Figs. 4 and 5), there also are fewer data values (n) for the extreme periphery, which causes the statistical variance to be greater. Because the adhesive strength test is destructive and only one sample can be obtained from a given eye, further analysis of the data is limited. We considered all eyes to be independent because we could not adequately study the statistical correlation between fellow eyes.

The polynomial equation given above was also used to fit a second-order regression line to the mean adhesive strength values in Figure 6. Table 2 lists the polynomial regression coefficients that describe this second-order function. Note that the second-order regression line is well-fitted to the data (r = 0.99),...
Table 2. Mean interlamellar adhesive strength function coefficients

<table>
<thead>
<tr>
<th>Second order polynomial function:</th>
<th>( Y = a_0 + a_1X + a_2X^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regression coefficients:</td>
<td>( a_0 = 15.90 )</td>
</tr>
<tr>
<td></td>
<td>( a_1 = -0.12 )</td>
</tr>
<tr>
<td></td>
<td>( a_2 = 0.59 )</td>
</tr>
<tr>
<td>Determination coefficient</td>
<td>( r = 0.99 )</td>
</tr>
</tbody>
</table>

excluding the central value, which lies below the line. This central deflection in the mean adhesive strength was also noticeable in many, but not all of the individual data plots, as shown in the examples of Figures 4 and 5.

The value at the very center of the cornea may actually be lower than the value we have measured with our technique, because of the use of a sample whose width is relatively large. Thus, even at the very center of the adhesive strength function, noncentral influences from the superior and inferior portions of the 1.8-mm-wide strip also are in effect.

Plots of adhesive strength at 0-mm corneal arc length versus age and time after death for all corneas were constructed to determine if these parameters influenced the results (Figs. 7, 8). No significant effect was observed within the range of data available. It should be noted that the loss of proteoglycans is not to be expected when the cornea is maintained in a moist chamber environment. Nevertheless, it would be misleading to conclude that storage time and other storage media variables would not have an observable effect on adhesive strength if studied under carefully controlled conditions.

**Histology**

In order to confirm the tearing depth and to examine the structural details at the tearing interface, histologic sections were produced from a few of the final corneas tested (Figs. 9–12). As shown in Figure 9, the tearing plane was at approximately 50% depth, and the torn ends of collagen lamellae were visible along the tearing plane interface in this midperipheral section.

The central region of the corneal strip showed no evidence of torn lamellae, but rather had a smooth interlamellar tearing interface (Fig. 10). This area corresponded to the central mean adhesive strength plot where the regression line does not predict the mean value.

Toward the peripheral regions, the tearing interface became more irregular in appearance, with increasing numbers of free ends of torn lamellae visible (Fig. 11). This region corresponded to the midperipheral portions of the adhesive strength function approximately 2 mm from the central cornea. It often was possible to trace the path of the torn lamellae back into the stroma. These lamellae had a distinct change in depth and orientation relative to the majority of the surrounding lamellae, so that they traveled obliquely across the tearing interface. Presumably, these lamellae would behave as anchoring fibers as the sample was torn apart, and thus would contribute their ten-
Discussion

Our results show that interlamellar adhesive strength varies with location in the human cornea, and histologic evidence suggests that at least two different structural mechanisms can account for these results. First, proteoglycan complexes within and between collagen lamellae provide a molecular level of binding, and second, binding strength is provided by the tensile strength of interwoven collagen lamellae that increase in number toward the periphery. Because of the lack of lamellar tearing in the central cornea, the adhesive strength value for this region may reflect primarily the molecular binding strength of the human cornea. The adhesive strength values of more peripheral regions may reflect the combined strength of molecular binding and lamellar tensile strength, or the lamellar tensile strength alone, depending on whether the properties behave in a serial or parallel manner.
Fig. 10. Histologic section from the posterior tearing layer of the central cornea. This smooth tearing interface with no torn lamellae occurred only in the central cornea, where adhesive strength was at a minimum. Bar = 50 \mu m.

Fig. 11. Thick section from the midperipheral cornea. Note the torn lamellae attached to the anterior tearing layer (above). Torn lamellae can be traced back into the anterior stroma and seen to vary in depth relative to the tearing plane. Bar = 50 \mu m.
Maurice and Monroe\textsuperscript{8} proposed some possible implications of molecular binding and lamellar interweaving as it related to their rabbit cornea results and to the biomechanical properties of shear strength. Their premise is directly applicable to the human cornea, which distinctly demonstrates the effects of these two mechanisms. As explained theoretically in their paper, the magnitude of shear strain would be diminished either by increased lamellar interweaving or by the limited ability of fibrils to slide with respect to one another due to the binding properties of the molecular matrix in which they are embedded.\textsuperscript{8} Thus, a greater degree of lamellar interweaving or stronger molecular binding would result in a higher shear strength and lower shear modulus.

Human corneas do have a higher shear strength than the rabbit cornea,\textsuperscript{10} as common experience confirms. For example, one can more easily slide between the finger and thumb the anterior and posterior surfaces of the rabbit cornea with respect to each other, than the human cornea, indicating structural translamellar binding differences between the two species.

The results of our study on interlamellar binding strength support the basis for greater shear strength of the human cornea. The central adhesive strength value of 14.2 g-wt/mm is larger than the value of 10 g-wt/mm reported by Maurice and Monroe,\textsuperscript{8} and may reflect stronger molecular binding in the human stroma. More significantly, the human interlamellar adhesive strength increased peripherally. Maurice and Monroe noted an increase towards the limbus in some of the rabbit corneas, but apparently did not find the predictable shape of the human data.\textsuperscript{8} We believe this to be an extremely important species difference that reflects the greater translamellar and interlamellar binding strength of the human cornea.

These results, if confirmed by studies of localized shear strength, may significantly alter our interpretation of and justification in comparing certain types of rabbit and human corneal data. In particular, rabbit models of the cornea that use biomechanical manipu-
lation in the form of surgical intervention and wound healing may need to be reinterpreted before directly applying the results to the human cornea.

Key words: corneal biomechanics, corneal collagen, corneal proteoglycans, corneal strength, corneal stroma

Acknowledgments

The authors gratefully acknowledge the assistance of Chiron Ophthalmics, Inc. for providing the recording and measurement devices used in this study.

References