A New Model for Evaluating Corneal Wound Strength in the Rabbit

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Presented in this report is a new model to evaluate corneal wound strength quantitatively by using an Instron 1125 tensiometer with video monitoring. Twenty-six rabbits received full thickness 8 mm central corneal incisions that were marked with sutures at each end and dressed with collagen shields. Animals were killed and corneas were harvested from 3-30 d postoperatively. Tensile test specimens, of rectangular (n = 9) or hourglass (n = 17) planform geometry, were prepared. Uniaxial tension tests were performed on these specimens parallel to the long dimension and perpendicular to the incision. Specimens were processed for light microscopy. Individual specimen load and displacement records were normalized to yield geometry independent stress and strain data sets. Maximum wound strength was determined as peak tissue stress on loading. Maximum tissue stiffness (inverse compliance) was derived from the peak slope of the stress-strain profile. Tissue fracture toughness was calculated as the total area under the stress-strain profile from the onset of loading to total wound failure. Data points at 3, 5, 10, 18, and 30 d show maximum wound strength increasing from 15.2 to 832 kilopascals (KPa), maximum stiffness increasing from 0.20 to 11.4 megapascals, and fracture toughness increasing from 1.82 to 87.7 KPa. Histological observations correlate well with tensiometry deductions. These data suggest enhanced mechanical load bearing with time, indicative of enhanced collagen fiber formation and cross-linking. Invest Ophthalmol Vis Sci 33:1727–1733, 1992

Wound healing is an important issue in corneal surgery. Variations in healing can affect the ultimate astigmatic refractive error. Also, the strength of the wound will affect patient activities during the recovery period.

A number of reports have evaluated the effect of various drugs and surgical techniques on the strength of corneal wounds.1-12 Two basic methods of wound testing have been employed. One method involves inserting a cannula into the eye and inflating the globe with air or saline.1,2 The pressure inside the eye is recorded at the point where the wound bursts. The second method involves preparing a rectangular strip of cornea with the incision perpendicular to the long axis. One end of this strip is fixed, while the other end is attached to an apparatus on which a series of weights is placed.3-10 Weights are added and recorded so that the applied load controls specimen elongation until the wound breaks catastrophically.

In the present study, tensile tests are performed using an Instron Model 1125 Universal Testing Instrument (Instron Corp., Canton, MA). This tensiometer operates in displacement control so that load varies in response to the resistance of the specimen to stretch. In this manner, the entire load-displacement signature of the specimen is observed, including wound fracture initiation and propagation. Ultimate load bearing and total energy absorption during deformation of the tissue is seen easily from strip chart and simultaneous video records of load versus deflection.

Materials and Methods

All animals used in this study were treated in accordance with the ARVO Resolution on the Use of Animals in Research.

Twenty-six adult female New Zealand white rabbits were anesthetized using accepted amounts of ketamine and xylazine intramuscularly. After the animals were found to be appropriately anesthetized, nictating membranes were removed bilaterally. With the aid of a Zeiss operating microscope (Carl Zeiss, Inc., Thornwood, NY), 8-mm central corneal incisions were made bilaterally using a sapphire knife. A pair of 10-0 nylon sutures was placed 1 mm from each...
end of the incision to serve as markers for the incision length and aid in specimen preparation on testing. The wound was not sutured to allow direct comparison with subsequent growth factor wound modality drugs, thus eliminating a variable. The wounds were dressed with 24 hr collagen shields that were pre-soaked in gentamycin ophthalmic solution. The shields were allowed to dissolve and were not replaced. Topical gentamycin was administered three times a day for a maximum of 10 days. All eyes were checked in room light three times a day for evidence of infection or wound dehiscence. Animals were killed at 3, 5, 10, 18, and 30 d postoperatively, and the corneas were harvested.

Test specimens were cut from the corneas into rectangular (n = 9) or hourglass (n = 17) shapes. All specimens possessed nominal lengths of 15.0 mm and were prepared so the incisions were perpendicular to the long planform dimension. Rectangular specimens were prepared using two blades 3.5 mm apart. Hourglass specimens were prepared using an 11 mm trephine so a minimum specimen width of 5.0 mm (nominal) occurred at the incision. Small metallic spheres were attached with cyanoacrylate glue flanking the wound to serve as points of reference for quantitative measurements. Specimens then were placed in the Instron 1125 tensiometer, with the incisions running horizontally, and underwent vertical uniaxial tension tests. The tensiometer was run in displacement control at the rate of 10 mm per min. Tension was measured via a load cell in series with the upper grip of the tensiometer. Load cell output produced a voltage proportional to the applied force and was recorded versus specimen extension on a strip chart. In addition, the load cell output voltage was superimposed on a videotape record of the deformation time course. The data from the strip chart and videotape were used to calculate tissue geometry, maximum wound strength, tissue stiffness, and tissue fracture toughness. Because specimen geometry strongly influences load-deflection profiles, stress and strain data were generated by normalizing load and deflection data by initial wound cross sectional area and initial specimen length, respectively. Maximum wound strength was taken as the peak tissue stress on loading.
Maximum tissue stiffness, which is the inverse of compliance, was derived from the peak slope of the stress-strain profile. Tissue fracture toughness was taken as the total area under the stress-strain profile from the onset of loading to total wound failure.

Incision sections that did not undergo tensiometry were processed for light microscopy (fixed in 2% glutaraldehyde/paraformaldehyde, dehydrated in alcohol and plastic embedded) and subsequent histological evaluation of the wounds.

**Results**

A schematic diagram of the testing apparatus is depicted in Figure 1. The long, slender specimen is secured by a pneumatically actuated grip at each end (Fig. 2)—the lower grip is fixed to the load frame while the upper is attached to a movable cross head. Slack length in the specimen is removed by a nominal preload, and initial specimen length (as measured between grips) and width (at the wound) are recorded. Loading of the sample is accomplished by grip separation brought about by cross head translation at the preselected speed. A typical load deflection record is

![Load deflection history for a 10-day specimen.](Fig. 3)

**Fig. 3.** Typical load deflection history for a 10-day specimen. The mechanical parameters used for comparison in the study are defined in the Figure. Cross-sectional area and gauge length of the specimen are geometric properties measured after application of a nominal preload prior to test initiation. Peak stress, peak tangent modulus, and fracture toughness measures represent maximum load bearing, peak stiffness (minimum compliance), and total elastic energy imparted to the tissue, respectively. By definition, these parameters are geometry-independent measures of mechanical strength.

![Cornea Tensiometry - PBS Control](A)

**Fig. 4.** (A) Peak stress vs. time. (B) Peak tangent modulus vs. time. (C) Tissue fracture toughness vs. time.
depicted in Figure 3, with load along the ordinate (from bottom to top) and deformation (change in specimen length) along the abscissa (from right to left).

Tensiometry data are shown in Figures 4a–c. As the healing time increased from 3–30 d, the peak stress of the wounds increased from 15.2 kilopascals (KPa) to 832 KPa (Fig. 4a). The peak tangent modulus, or maximum tissue stiffness, increased from 0.20 megapascals (MPa) to 11.4 MPa (Fig. 4b). Tissue fracture toughness increased from 1.82 KPa to 87.7 KPa (Fig. 4c). There is a positive correlation between increases in these measurements and postoperative time.

Three, ten, and thirty day wounds were sectioned for light microscopy. At 3 d, the wound is still mostly open (Fig. 5). There is some closing posteriorly with evidence of some endothelium and fibrin filling the area. There is an apparent continuity of the endothelial side. The remainder of the wound is lined with epithelium and there is evidence of some inflammatory cells. At 10 d (Fig. 6), the wound has completely closed and appears to be bridged by collagen fibrils in a mostly irregular pattern with areas of increased organization. The epithelium is limited to the ocular surface. At 30 d (Fig. 7), the wound is more organized. Collagen fibrils fall into the regular lamellar pattern.

**Discussion**

The displacement control ability of this model affords certain advantages to the study of wound strength over previous models that used load control. Load control models that use sequential dead-weight loads incorporate "dynamic" effects that could result in premature catastrophic wound failure, thereby underestimating maximum load bearing. Our model instead allows for a continuous application of deformation and load. Wound failure is controlled, allowing for the quantification of tissue fracture and energy absorption throughout the deformation/failure process. We are able to obtain full deflection-load profiles from preloading to complete failure of the wound. As such, stiffness and toughness measures are possible in addition to stress and load bearing information docu-
Fig. 7. Light microscopy section of a 30-day wound. Note the more organized lamellar pattern (X288).

mented in earlier studies. Numerous wound failures were progressive in nature. That is, a specific site of fracture initiation was observed in many specimens with subsequent expansion of the wound dehiscence. Although catastrophic failure modes were observed in several specimens, the progressive failure mode predominated. Such activity may suggest an uneven collagen cross-linking and synthesis at the wound.

The use of two different specimen planforms in this study deserves further explanation. Early pilot studies, which generated the 5-d and 18-d data reported here, used rectangular planform specimens. Such an approach is consistent with standard engineering materials testing procedure. However, excised corneas needed to be flattened for rectangular specimen dissection, thereby stretching the cornea endothelium and subendothelium when inherent concavity of the cornea is removed. Although specimen curvature is removed when loaded by the Instron tensiometer, the potential for some wound dehiscence during specimen preparation could not be ruled out. To avoid this potential prestretch of wound endothelium, the hourglass planform used to generate the 3, 10, and 30 d results was adopted. Dissection protocol for hourglass specimens did not remove cornea concavity until just prior to insertion into the Instron tensiometer.

Data analysis for the rectangular and hourglass planform specimens was based on the assumption of a uniform stress distribution in the vicinity of the wound. Figure 8 depicts contours in load-directed stress for each specimen planform as determined from finite-element analysis. The contour legend is depicted in each figure and has units of grams per square millimeter. The numerical finite-element analysis employs a nominal load applied to each specimen so that a stress level of 2 g/mm² should occur at mid-length. Figure 8a clearly shows that rectangular specimens possess uniform stress levels in the wound region. Hourglass specimen stress distribution is not uniform, as seen in Figure 8b, and possesses a distinct stress concentration at the lateral edges of the wound. The maximum stress magnitude is 2.301 g/mm² and represents a 15% elevation over the desired value of 2 g/sq². Hourglass specimens will, therefore, initiate failure at the stress concentration site. If the stress distribution were uniform, failure would presumably occur evenly across the wound at a load value 15% higher than that indicated by our data. Thus, peak stress, peak tangent modulus, and fracture toughness data reported at 3, 10, and 30 d may be underestimated by approximately 15%. The time course data depicted in Figure 4 of a monotonic rise, plateau, and subsequent monotonic rise in wound strength post-operatively remains qualitatively correct if hourglass planform stress concentrations are considered.

The data presented in this study confirm that as wounds heal over time, increases in wound tensile strength, maximum stiffness, and tissue fracture toughness occur. This suggests enhanced mechanical load bearing indicative of enhanced collagen fiber formation and cross-linking. Histological studies substantiate these findings. Epithelial and fibrin plugs initially filling the wounds are slowly replaced by new, progressively more organized stromal components. The triphasic time history of wound stress, elastance, and fracture toughness depicted in Figure 4 may reflect distinct phases of corneal tissue repair. Inflammation, granulation tissue formation, and tissue remodelling have been identified as successive healing phases during analogous cutaneous tissue repair. The apparent plateau in corneal wound strength indicators (depicted in Figs. 4a-c) may reflect a transition from fibroplasia to collagen synthesis and, therefore, demark early and late wound healing phases. This new experimental approach to the study of corneal wound repair provides data of a quantitative nature that can be stored, retrieved, and statistically analyzed. The procedure seems ideal for objec-
Fig. 8. Stress contour plots for rectangular planform (A) and hourglass planform (B) specimens loaded uniaxially. Axial specimen direction is the horizontal figure direction. Each planform shape is subject to a clamped left edge and uniform tensile loading along the right edge. Developed stress in each specimen is determined via finite-element analysis. Stress data are given in units of g/mm². For illustrative purposes, an applied load is prescribed that should yield a nominal stress of 2 g/mm² at mid-span (i.e., the wound location in actual cornea test specimens). The divergence of hourglass stress levels from the desired is indicative of stress concentration phenomena that lead to premature specimen failure.

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