A Mechanical Model of the Cornea Considering the Crimping Morphology of Collagen Fibrils

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Purpose. To develop a mechanical model with which to investigate the relationship between the crimping morphology of collagen fibrils and the nonlinear mechanical behavior of the cornea.

Methods. Uniaxial tensile experiments were performed with corneal strips to test their mechanical behavior. A constitutive model was constructed based on the Gaussian-distributed morphology of crimped collagen fibrils. The parameters that represent the micro characteristics of collagen fibrils were determined by fitting the experimental data to the constitutive model. Transmission electron microscopy (TEM) was used to visualize the crimping morphology of collagen fibrils in the stroma. A quantitative analysis of fibril crimping degrees in the TEM images was conducted to test the parameters predicted by the constitutive model.

Results. The parameters were derived using a fitting method that included the expectation for the distribution of fibril crimping degrees, $\mu = 1.063$; the standard deviation, $\sigma = 0.0781$; the elastic modulus of collagen fibrils, $E = 52.74$ MPa; and the fibril ultimate strain, $\varepsilon_b = 0.1957$. TEM images showed a variation of the fibril crimping morphology when the cornea was subjected to different tensile loads. A good agreement was found between the parameters derived by the constitutive model and the data quantified from the TEM images.

Conclusions. The nonlinear mechanical behavior of the cornea is closely correlated with the crimping morphology of collagen fibrils. The findings are expected to guide further research of corneal pathologies related to the abnormal microstructure of collagen fibrils.

Keywords: fibril crimping morphology, constitutive model, corneal mechanical properties

The stroma, which accounts for approximately 90% of the corneal thickness, determines the mechanical properties of the cornea. The stroma is formed by more than 200 successive lamellae of collagen fibrils embedded in a hydrated proteoglycan matrix.[1,2] In the anterior stroma, collagen fibrils are strongly isotropic due to lamellar interweaving, whereas in the posterior stroma, most fibrils are arranged preferentially.[3–5] X-ray scattering studies revealed that fibrils have a preferential orientation in the superior-inferior and nasal-temporal directions.[6–8] Knowledge of the relationship between the organization of collagen fibrils and the mechanical properties of the cornea is important for understanding pathologies of corneal diseases related to abnormal microstructure such as keratoconus.[9–11]

The pioneering corneal model considering micro characteristics of the stroma was introduced by Pinsky and Datye.[12] Recently, based on the distribution and organization of collagen fibrils, several models have been introduced to describe corneal anisotropic behavior.[13–18] Also, an inverse numerical analysis has been performed to determine mechanical parameters of the cornea by using an inflation test.[19] A more recent study reported a constitutive model that, for the first time, accounts for three-dimensional, inclined lamellae and their depth-dependent distribution.[20]

Strongly nonlinear stress-strain behavior, or hyperelasticity, is an important mechanical behavior of the cornea, which is also considered to be related to fibril microstructure. Grytz and Meschke[21,22] proposed a constitutive model to investigate the interaction between the fibril morphology in the stroma and the mechanical conditions of the cornea. This model was based on the hypothesis that collagen fibrils in a load-free state are crimped like a helical spring.[23,24] The spring, under a tensile force, was used to represent mechanical behavior of the fibrils. Studies related to fibril crimping morphology were also conducted by Lanir,[25,26] Zulliger et al.,[27] Hurschler et al.,[28] and Cacho et al.[29]

In this study, we introduced the idea of "crimping degree" to represent the level of crimping of the collagen fibrils. The crimping degree is defined as the ratio between the straightened length and the original crimped length of a fibril. In the cornea, there are a large number of collagen fibrils lying in the stroma. In a load-free state, the individual fibril is crimped at

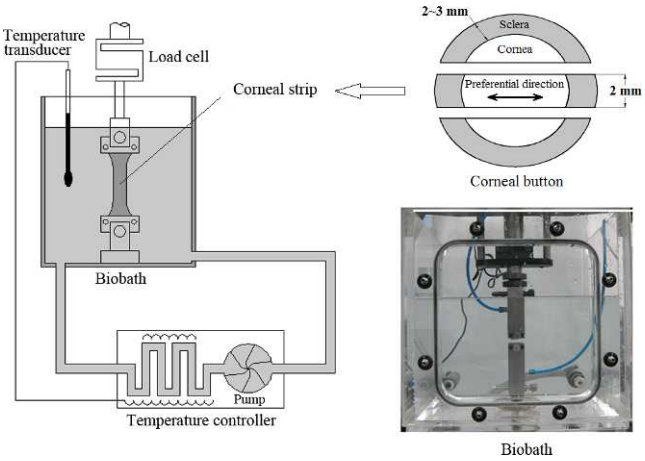

FIGURE 1. Schematic representation of uniaxial tensile test of corneal strips.

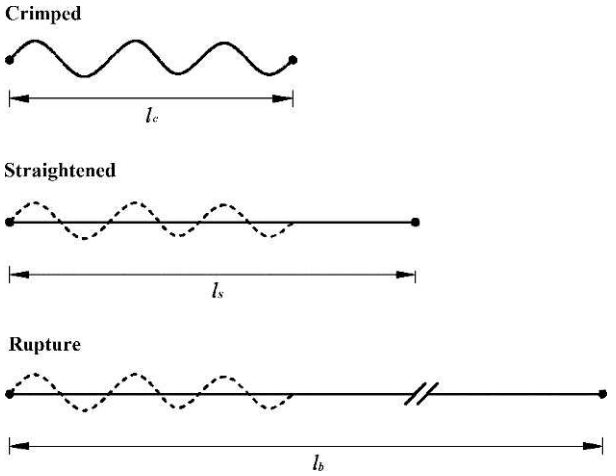

FIGURE 2. Diagram of the shape of a single fibril in 3 states: crimped, straightened, and broken.

different degrees. The distribution of crimping degrees is assumed to follow a Gaussian function. When the cornea is subjected to a tensile load, each fibril undergoes an independent deformation. The straightened fibrils, acting as reinforcing frames, enhance the corneal mechanical properties until they break. We postulated that, if the effect of water and proteoglycans is not considered, the crimping morphology of collagen fibrils is responsible for the highly nonlinear mechanical behavior of the cornea.

In the current work, a constitutive model is presented to investigate the relationship between the crimped morphology of collagen fibrils and the mechanical properties of the cornea. First, the nonlinear stretch-stress relationship of the cornea was derived by uniaxial tensile tests. Then, the parameters that represent the fibril crimping morphology were determined by fitting the experimental data to the constitutive model. Using transmission electron microscopy (TEM), we imaged crimping morphologies of the fibrils under different load conditions. Quantification of the crimping degrees was conducted to testify the predictions made by the constitutive model.

METHODS

Uniaxial Tension Tests

The study was approved by the local research and ethical committees, and the research adhered to the tenets of the Declaration of Helsinki. Bovine cornea was selected as the subject specimen because collagen fibrils in the central region are arranged dominantly in 1 preferential (inferior-superior) direction.[30] It can diminish the mechanical interference caused by the fibrils in nonpreferential directions. Twenty-four enucleated bovine eyes were obtained from a local abattoir within 4 hours postmortem. The entire cornea with adjacent sclera (~2–3 mm width) was then extracted from each eye (Fig. 1). To maintain corneal hydration without swelling, the specimens were preserved in Eusol C (Alchimia, Padova, Italy) within 4 hours before the mechanical tests. An experimental test was conducted to analyze corneal swelling in different solutions (see Figure S1 in Supplement 1). The result indicated that the corneas showed no swelling when they were kept in Eusol C during that time, which is also supported by previous studies.[37,38] Using a self-designed double-bladed scalpel, we obtained a 2-mm-wide limbus-to-limbus strip centered on the cornea, along the inferior-superior direction, from each specimen. Mechanical tests were conducted using a material testing machine equipped with a load cell capable of 50 newtons (N; 800LE mechanical test system; TestResources, Inc., Shakopee, MN, USA). The strip was connected to a pair of clamps with a length of approximately 3 to 4 mm on both sides, so that only the central region of the strip was tested. To prevent dehydration, corneal strips were kept in a water bath system (BioBath environmental chamber; TestResources) filled with Eusol C solution. Uniaxial tensile tests were performed with the corneal strips with a speed of 1 mm/min until the breaking point was reached. A schematic representation of the uniaxial tensile test is shown in Figure 1. As the tests were conducted in solution, it is important to note the buoyancy effect, which would influence the measurements. To obtain more accurate data, a nonload test in solution was conducted before each uniaxial test. The final results eliminated the buoyancy effect by subtracting the nonload measurements from the load elongation measurements.

Constitutive Modeling

Previous studies have revealed that stromal collagen fibrils are crimped with a loose configuration in the load-free cornea.[21,22] Accordingly, a constitutive model was introduced to describe the fibril morphological change under different load conditions. The modeling was based on an important assumption: the cornea is composed of collagen fibrils that lie only in the preferential (tensile) direction. This assumption is supported by studies by Meek et al.[3,6] that show collagen fibrils in the posterior stroma are arranged preferentially. Contributions by other components and the interaction between the neighboring fibrils are not considered in the model.

In a load-free corneal strip, the fibril has a wavy shape with a crimping degree, d_c, which is defined as the ratio between the straightened length l_s and the crimped length l_c of a fibril: $d_c = l_s/l_c$. When the corneal strip is stretched, a single fibril experiences 3 phases: a crimped, straightened, and broken phase (Fig. 2). As the fibril is crimped, it is unable to resist the tension due to its slack shape. With elongation of the corneal strip, the fibril crimping degree decreases gradually. When the fibril is straightened, it has the capability of tension resistance and this contributes to the mechanical reinforcement of the cornea. The straightened fibril follows Hooke's law. Once the load on the fibril exceeds the tensile strength, ε_b (maximum strain), the fibril will break immediately and never resist any tension (Fig. 2). The relationship between the stretch of a corneal strip, Λ, and the tensile stress of a single fibril, t, can be

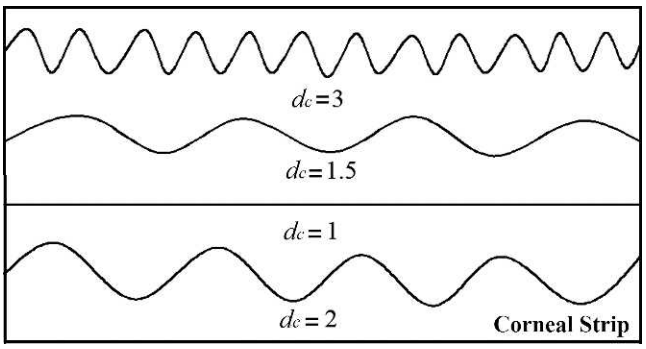

FIGURE 3. A corneal strip is composed of collagen fibrils crimped at different degrees.

written as:

$$t = \begin{cases} 0, & \Lambda < d_c \\ e(\Lambda/d_c - 1), & d_{crimp} \ll \Lambda < d_c(1+\varepsilon_b) \\ 0, & \Lambda \gg d_c(1+\varepsilon_b) \end{cases} \quad (1)$$

where the stretch parameter Λ is defined as the ratio between the elongated length, L, and the initial length, L_O, of the corneal strip. Variable e is the elastic modulus of the fibril. More details of the mathematical development of Equation 1 can be found in Appendix A.

In the stroma, there are a large number of collagen fibrils characterized by various crimping degrees (Fig. 3). We assumed that the crimping degrees followed a Gaussian distribution in a load-free corneal strip. Its probability density function can be expressed as:

$$f(d_c, \sigma, \mu) = \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{d_c-\mu}{\sigma}\right)^2} \quad (2)$$

where parameters μ and σ are called expectation and standard deviation, respectively, which together represent the characteristics of a Gaussian distribution.

When a corneal strip is subjected to a tensile load, only straight fibrils are responsible for the load resistance. When the strip is elongated, more and more fibrils are straightened to resist tension. Thus, the distribution of fibril crimping degrees determines the corneal nonlinear stress-stretch relationship. When the corneal strip is stretched to Λ, the corneal stress, T, can be given as an integral of the fibril crimping degrees:

$$T(\Lambda) = e\int_a^b (\Lambda/d_c - 1) \cdot \frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{\lambda_0-\mu}{\sigma}\right)^2} \cdot \mathrm{d}(d_c) \quad (3)$$

where

$$a = \begin{cases} 1, & \Lambda < 1+\varepsilon_b \\ \Lambda/1+\varepsilon_b, & \Lambda \geq 1+\varepsilon_b \end{cases}, \quad \text{and } b = \Lambda \quad (4)$$

More details of the development of Equation 3 and the integral boundaries in Equation 4 can be found in Figure 4 and Appendix B.

Fibril Crimping Morphology Observation

Another 24 corneal strips with the same size as the strips used in the tensile tests were prepared for the purpose of observing the crimping morphologies of collagen fibrils under different load conditions. For the load-free condition group, 6 strips were initially kept in Eusol-C for 2 hours in order to remove their internal stress. A 2.5% glutaraldehyde solution was then used to fix the fibril morphologies in load-free strips. For the loaded conditions, 18 strips were equally divided into 3 groups, which were subject to 3 different tensile stretches (Λ = 1.1, Λ = 1.2, and Λ = 1.3) provided by the testing machine. The strips were not mounted to the clamps directly but were connected with a pair of self-designed holders (Fig. 5). When the required stretch was reached, the holders were locked to hold the current strip elongation. The samples with the locked holder were then immersed in the fixative solution for no less than 2 hours. After fixation, the central region of each strip was cut into 5 segments. An ultrathin film was made from a transverse (or horizontal) plane of the posterior stroma parallel to the posterior surface of the cornea. We used a transmission electron microscope (JEM-1400 model; JEOL Ltd., Tachikawa, Japan) to observe the fibril crimping morphology at a micro level. In each film, collagen fibrils were derived from 20 randomly selected regions for quantifying the crimping degrees. It should be noted that the distance between the ultrathin film and the posterior surface was approximately one-third of the total corneal thickness. Although it was not a requirement for accuracy for the distance, all the ultrathin films were close to the posterior surface. The fibril crimping degrees were calculated using AutoCAD 2008 software (Autodesk, Inc., San Rafael, CA, USA). This software enables capture of the tops of the fibril wave and measure the curve length as well as the distance between the 2 tops automatically. A quantitative analysis was performed using SPSS version 17.0 software (SPSS, Inc., Chicago, IL, USA).

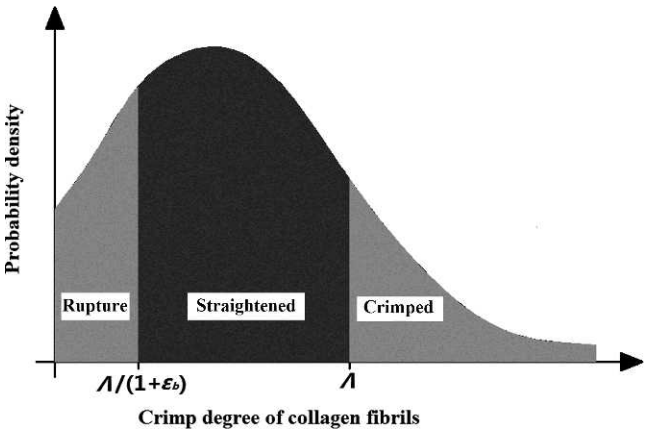

FIGURE 4. Integral boundaries of the constitutive model for the distribution of the fibril crimping degrees.

RESULTS

Figure 6 shows the average stress-stretch curve of the cornea from 24 uniaxial tensile tests. The typical curve can be empirically divided into 3 regions[31]: the O-A region ("toe" region) is characterized by stress increasing nonlinearly with stretch, where σ_A = 1.492 ± 0.431 MPa, and λ_A = 1.096 ± 0.017. The A-B region shows a strong linearity of the stress-stretch curve, whose slope is the elastic modulus of the cornea, where E = 34.113 ± 6.14 MPa. Point B is the endpoint of the linear region, where σ_B = 4.895 ± 0.860 MPa, and λ_B = 1.226 ± 0.028. The B-C region, named the "heel region," presents a nonlinearly shaped area. At point C, the corneal strip ruptured, where σ_C = 5.513 ± 0.907 MPa, and λ_C = 1.224 ± 0.033, which is the corneal tensile strength.

Figure 7 illustrates the constitutive model fitting to a set of corneal stress-stretch data from 1 mechanical test. The slight difference between the experimental data and the fitting curve indicates that the constitutive model can reproduce the tensile

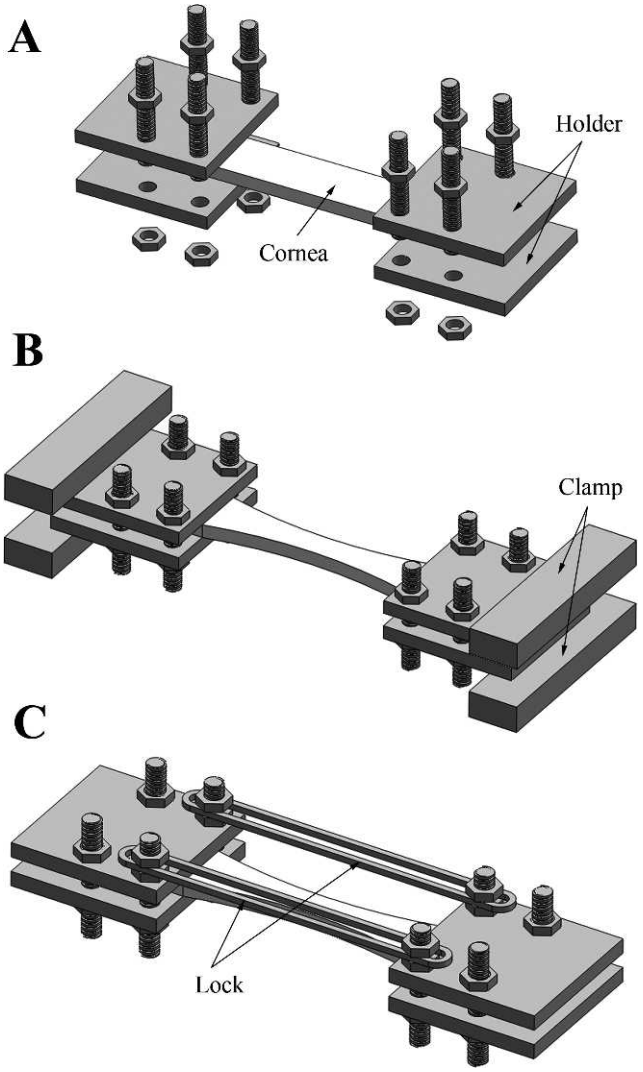

FIGURE 5. A pair of holders is used to fix the corneal strips prepared for the TEM observations. (**A**) A corneal strip is mounted onto the holders; (**B**) the holders are fixed to the crimps in a strip test; (**C**) the temporal shape of the corneal strip is held by locking the holders when the acquired stretch is reached.

test well. The parameters that represent the micro characteristics of collagen fibrils were determined by being fitted to 24 sets of experimental data (Table). The parameters included the expectation, μ, and the standard deviation, σ, for the distribution of fibril crimping degrees, the elastic modulus E, and the ultimate strain, ε_b, of collagen fibrils. The expectation value, $\mu = 1.063$, indicates that most collagen fibrils appear to have a crimping degree of 1.063 under a load-free condition. The standard deviation, σ, represents the amount of dispersion away from the expectation. The parameters μ and σ together determine the shape of the probability density curve of the fibril crimping degrees (see Fig. 9). Note that the density curve is asymmetrical as it is redundant when d_c is ≤ 1. Thus, the value of the standard deviation, σ, provides an approximation of the data dispersion. E and ε_b represent the mechanical properties of collagen fibrils, which were determined by the characteristics of the corneal stretch-stress curve. The elastic modulus of the collagen fibrils was predicted to be 52.74 MPa, which is less than the order of magnitude (1.0 GPa) reported in previous studies.[32] In the constitutive model, the cornea strip is assumed to be fully composed of collagen fibrils. Therefore,

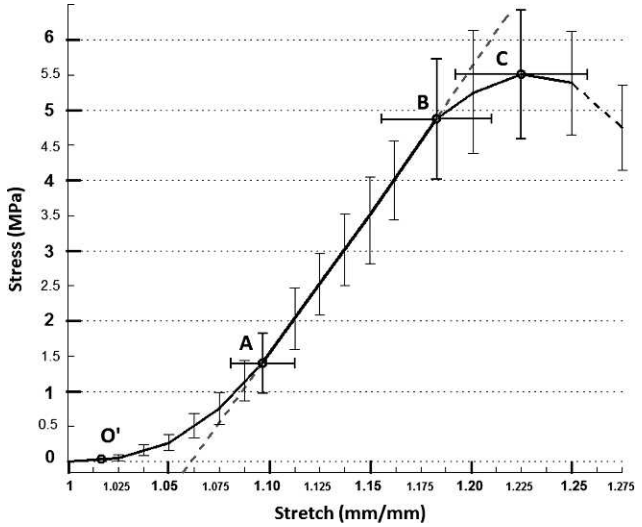

FIGURE 6. The average stretch-stress curve of the cornea was derived from 24 strip tests. The typical "J" curve can be divided into 3 phases: O-A (the "toe" region), A-B (the linear region), and B-C (the "heel" region).

the elastic moduli of the cornea and collagen fibrils are of the same order. The macro mechanical properties of the cornea have a strong influence on the parameters E and ε_b.

TEM images in Figure 8 show the variation of the crimping morphology of collagen fibrils when the corneal strips were subjected to 4 different load conditions. The collagen fibrils exhibited a significantly wavy shape in the load-free state (Fig. 8A). With strip elongation, the fibrils were reformed "uncrimped" gradually until they were straightened (Figs. 8B, 8C). At $\Lambda = 1.3$, some fibrils had been broken at the micro level, whereas the corneal strip did not rupture (Fig. 8D).

Figure 9 shows the distribution of the fibril crimping degrees in the load-free situation. Quantification of the crimping degrees indicates that the distribution produced a Gaussian function. Figure 10 shows the amount of the straightened fibrils under different load conditions. With strip elongation, more and more fibrils were straightened until they broke. A good agreement was found between the parameters

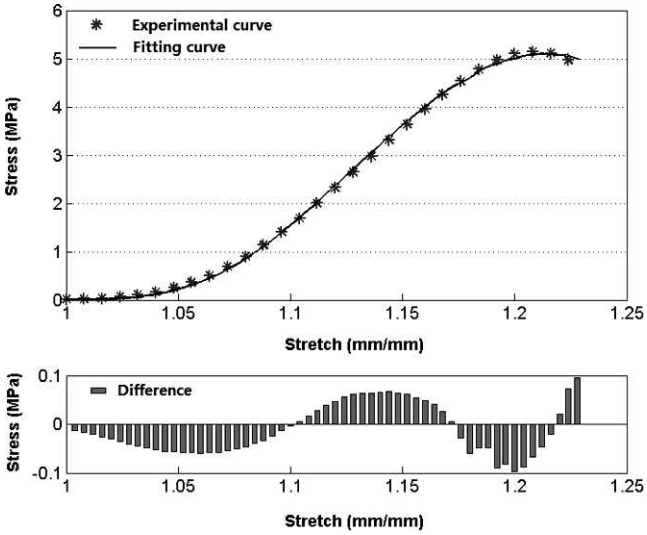

FIGURE 7. Fitting to a set of corneal stress-stretch data by the constitutive model.

TABLE. Parameters Representing Micro Characteristics of Collagen Fibrils Predicted by the Constitutive Model

Parameter	μ	σ	*E*, MPa	ε_b
Mean	1.063	0.0781	52.74	0.1957
SD	0.134	0.0144	8.39	0.0211

E, elastic modulus of fibrils; ε_b ultimate strain of collagen fibrils; σ, standard deviation for distribution of fibril crimping degrees; μ, expectation for distribution of fibril crimping degrees.

predicted by the constitutive model and the data quantified from the TEM images, which indicates that the nonlinear mechanical behavior of the cornea is closely correlated with the crimping morphology of collagen fibrils.

DISCUSSION

This paper presents a microstructural constitutive model used to investigate the relationship between the crimping morphology of collagen fibrils and the nonlinear mechanical behavior of the cornea. Our work was based on the important prerequisite that the cornea consists of collagen fibrils that are arranged solely in the preferential direction. Actually, collagen fibrils are arranged preferentially only in the posterior two-thirds of the stroma. In the anterior stroma, the fibrils are more isotropic due to lamellar interweaving. However, lamellar interweaving has little influence on mechanical properties of the corneal strip, because the inclined fibrils would probably be under a complete or partial load-free state when the strip is subjected to tensile load. The stroma is one of the 5 layers forming the human cornea. Mechanical properties of the cornea are determined primarily by the stroma as it accounts for 90% of the total thickness.[33] In the stroma, collagen fibrils are surrounded by a matrix of water and proteoglycan, which contributes little to the loading resistance in the strip test.[39] Effects of the ground matrix and its interaction with the collagen fibrils were not considered in the current study. Therefore, the preferential collagen fibrils in the stroma were primarily responsible for the mechanical behavior of the corneal strip. However, it should be noted that the hydrated matrix mainly determines the viscous (time-dependent) behavior of the cornea. Hydration and proteoglycan distribution should be accounted for when modeling corneal viscoelastic behavior.

Figure 6 shows that the stress-stretch curve of the cornea presents a J shape. This J-shaped curve can be divided into 3 regions[31]: the "toe" region, the linear region, and the "heel"

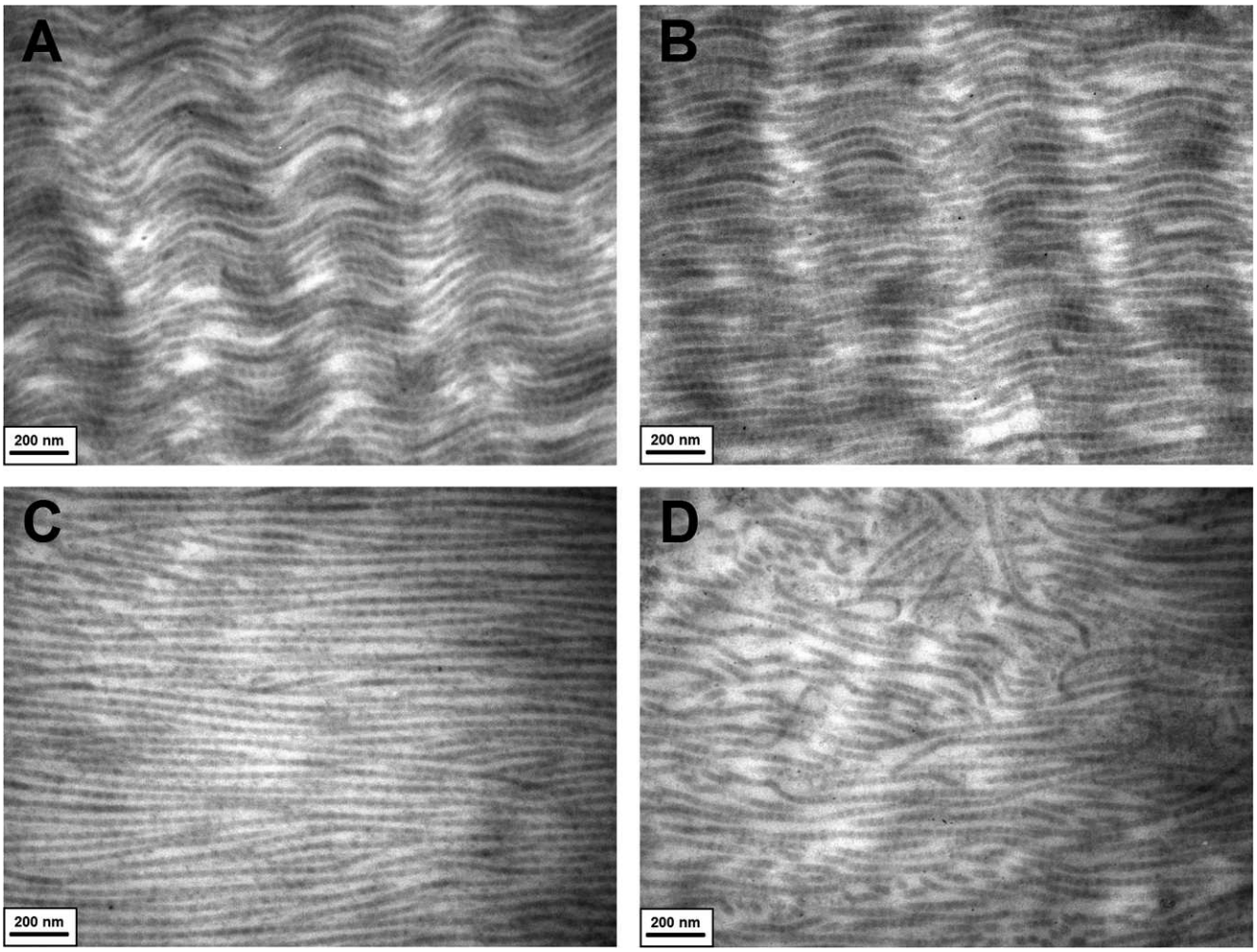

FIGURE 8. TEM images show a variation of the fibril crimping morphologies under 4 different load conditions. Under the load-free condition (Λ = 1.0), collagen fibrils exhibited wavy shape by a large degree of crimping (**A**). With the strip elongated, the fibrils showed as gradually "uncrimped" ([**B**], Λ = 1.1) until they were straightened ([**C**], Λ = 1.2). Some fibrils were found broken at Λ = 1.3, whereas the corneal strip had not ruptured (**D**).

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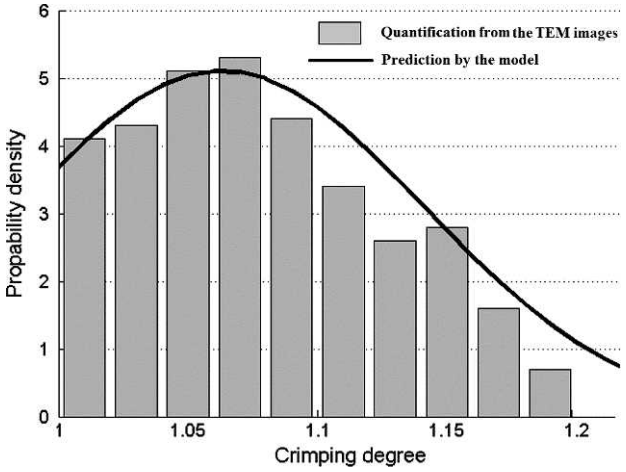

FIGURE 9. Comparison of the probability density of fibril crimping degrees between the prediction by the constitutive model and the qualification from the TEM images in the load-free corneal strips.

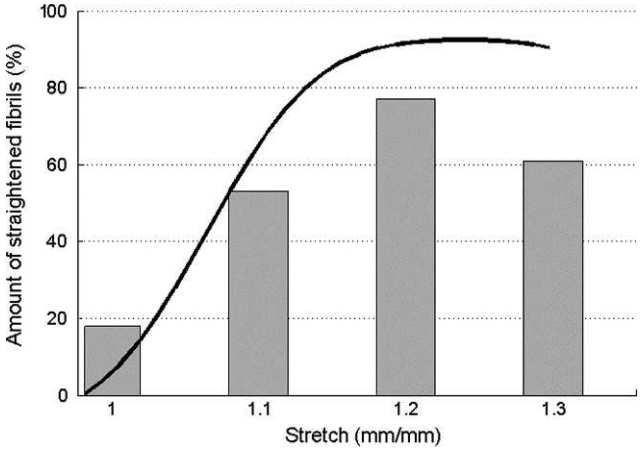

FIGURE 10. The amount of straightened fibrils under different load conditions.

region. In the "toe" region, the corneal stiffness (elastic modulus) grows quasi-exponentially with elongation. The TEM image shows that collagen fibrils exhibit a wavy appearance with high crimping degrees in the load-free strip (Fig. 8A). With the corneal strip elongated, the fibril crimping degrees decreased until the fibrils are straightened. When the fibril crimping degrees were presumed to follow a Gaussian distribution, the constitutive model could well reproduce the corneal stress-stretch curve in the tensile test (Fig. 7). This confirmed that the distributed crimped morphology of the collagen fibrils is responsible for the nonlinear behavior of the cornea, despite the fact that individual fibrils are linearly elastic.

In the linear region, the stress-stretch curve appeared to be strongly linear. TEM observation showed that most of the fibrils are straightened in this state (Fig. 8C). The straightened fibrils act as reinforced frames to enhance the corneal mechanical properties. Accordingly, the cornea has the greatest elastic modulus (the slope of the linear region) in this region. The corneal elastic modulus reported in the literatures ranges widely by more than 1 order of magnitude. The large differences between data could be mainly because of different experimental methods such as strip extensometry, inflation, and indentation tests.[34–36] Previous studies suggested that the corneal elastic modulus obtained by strip extensometry test is significantly larger than that obtained by inflation test.[37,40] Also, Elsheikh and Anderson[41] introduced a model to match the parameters obtained from both methods. However, even though the corneal elastic moduli were derived from strip extensometry tests, there are significant differences among the measurements. Many factors can be responsible for the large dispersion of the values, including the specimen origins, the level of strain, the testing protocol, and the calculation method. Hoeltzel et al.[42] and Zeng et al.[34,43] concluded that there is little difference in the mechanical properties of different origins. However, corneal elastic modulus under a large strain (~10–60 MPa) is much greater than that under a small strain (~0.1–3 MPa).[37,44] The experimental protocols such as the preservation solution,[45,46] the cycle number of the preloading,[37,41] and the testing environment (e.g., use of water bath or not)[43,47] make a great difference in the results. Some corneal elastic moduli in the literature are derived using a secant modulus, which is less than a tangent modulus in the linear region of the strain-stress curve.[37,48] Also, some results in the literature were obtained from a modified formula to overcome the effects of nonphysiological loading.[44,49]

In the "heel" region, the stress-stretch curve shows a slow ascent and, after the ultimate strength point C, a rapid descent. Once a single fibril is stretched to exceed its ultimate strain, it will break and will never bear a load. The TEM image shows that some of the fibrils were broken before the strip ruptured completely (Fig. 8D). This indicates that once the stress or stretch exceeds the upper limit of the linear region (Fig. 6, point B), the cornea would be physically damaged. A quantitative analysis of collagen fibrils in TEM observations indicates that the crimping morphology of collagen fibrils is distributed in a nearly normal fashion.

The constitutive model predicts a probabilistic crimping morphology of collagen fibrils under a load-free condition. Actually, in the naturally physiological condition, the contact cornea is not load free but is subjected to IOP. IOP leads to a state of isotropic stress in the central region of the cornea. To remove the internal stress, a corneal strip should be kept in a Eusol-C solution for 2 hours. However, even in a hydrated state, the collagen fibrils crimped in a load-free corneal strip would be significantly different from those in a load-free intact cornea. As mentioned earlier, there are a large number of nonpreferential or inclined fibrils in the cornea (especially in the anterior one-third of the stroma), which would be entirely or partly load free even in a loaded strip. Accordingly, the nonpreferential fibrils would probably have a higher crimping degree in a load-free strip. To obtain more accurate measurements of the fibril crimping degrees distribution, the statistical data were derived from the collagen fibrils only in the preferential (tensile) direction as well as in the posterior one-third of the cornea, which is consistent with the prerequisite for the modeling.

In conclusion, our work aimed to investigate the relationship between the micro morphology of collagen fibrils and the macro mechanical behavior of the cornea. The findings suggest that the Gaussian-distributed fibril morphology contributes to the cornea's highly nonlinear behavior. These findings are expected to guide future research of the corneal pathologies related to the abnormal microstructure of collagen fibrils.

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Appendix A. A Constitutive Model of a Single Fibril

For a crimped fibril, l_c and l_s are the initial crimped length and straightened lengths, respectively, of the fibril. The crimping degree is the ratio of l_c to d_c, where l_s/l_c. l_b is the length when the fibril is stretched just to the breaking point. ε_b is the maximum tensile train that a single fibril can bear, which can be written as:

$$\varepsilon_b = \frac{l_b - l_s}{l_s} \quad \text{or} \quad l_b/l_c = d_c(1+\varepsilon_b) \tag{A1}$$

A crimped fibril does not have tensile resistance. When the fibril is straightened, it follows Hooke's law until it breaks at the maximum strain point ε_b. Accordingly, when a corneal strip is stretched to L, the relationship between the elongation of the strip L and the tensile stress on a single fibril, t , can be written as:

$$t = \begin{cases} 0, & L<l_s \\ e(L-l_s)/l_s, & l_s \ll L<l_b \\ 0, & L \gg l_b \end{cases} \tag{A2}$$

where e is the elastic modulus of a fibril. Lowercase letters are used for parameters related to a single fibril at the micro level, whereas capital letters are used for parameters of a corneal strip at the macro level.

The stretch ratio of the corneal strip, Λ, was defined as the ratio between the extended length L and the initial length of a corneal strip: $\Lambda = L/L_0$. It should be noted that L_0 is equal to the initial crimped length of a single fibril: $L_0 = l_c$. Then, the Equation A2 can be rewritten as:

$$t = \begin{cases} 0, & \Lambda < l_s/l_c \\ e\left(\dfrac{L/L_0}{l_s/l_c} - 1\right), & l_s/l_c \ll \Lambda < l_b/l_c = \text{Eq.}(1) \\ 0, & \Lambda \gg l_b/l_c \end{cases} \tag{A3}$$

Appendix B. A Constitutive Model of Gaussian-Distributed Crimping Degrees

Degrees of fibril crimping in a load-free state were assumed to produce a Gaussian distribution, which is produced by a probability density function, f (d_c, σ, μ) (Equation 2). The graph of Gaussian probability represents a typical bell-shaped curve, which is characterized by 2 parameters: the mean μ and the standard deviation σ (Fig. 1A). When a corneal strip is stretched to Λ, the collagen fibrils of $d_{crimp} = \Lambda$ are just straightened. Without considering fibril break, the tensile stress produced in the cornea, T, is carried by all the straightened fibrils, ($d_{crimp} \leq \lambda$). Then, the integral ranges from 1 to Λ, which can be expressed as:

$$T(\Lambda) = e\cdot\int_1^{\Lambda} (\Lambda/d_c - 1)\cdot\frac{1}{\sigma\sqrt{2\pi}} e^{-\frac{1}{2}\left(\frac{\Lambda-\mu}{\sigma}\right)^2}\cdot \mathrm{d}(d_c) \tag{B1}$$

When considering fibril break, the lower limit of the integral should be modified, because the fibrils with $\varepsilon > \varepsilon_b$ cannot resist the tensile load. For a single fibril, its strain ε at the stretched length of a corneal strip, L, is:

$$\varepsilon = \frac{L - l_s}{l_s} \tag{B2}$$

When $\varepsilon > \varepsilon_b$,

$$\varepsilon = \frac{L/L_0 - l_s/l_c}{l_s/l_c} > \varepsilon_b \Rightarrow d_c < \frac{\Lambda}{1+\varepsilon_b} \tag{B3}$$

Therefore, when a corneal strip is stretched to Λ, the collagen fibrils of $d_c < \Lambda/(1+\varepsilon_b)$ have broken and lost their load resistance. Accordingly, the lower limit is mortified as $\Lambda/(1+\varepsilon_b)$. It should be noted that when $\Lambda < 1+\varepsilon_b$, the lower limit returns to 1 because the Λ is never less than 1.

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