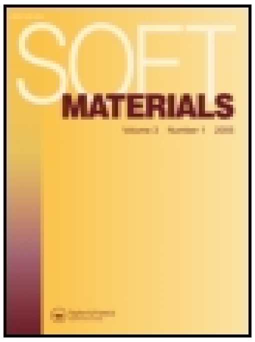

Soft Materials

ISSN: 1539-445X (Print) 1539-4468 (Online) Journal homepage: http://www.tandfonline.com/loi/lsfm20

Depth-dependent mechanical characteristics of Porcine cornea

Ruiqi Du, Hanjing Tian, Xianghong Xu, Yingfeng Shao & Fan Song

To cite this article: Ruiqi Du, Hanjing Tian, Xianghong Xu, Yingfeng Shao & Fan Song (2016): Depth-dependent mechanical characteristics of Porcine cornea, Soft Materials, DOI: 10.1080/1539445X.2016.1242497

To link to this article: http://dx.doi.org/10.1080/1539445X.2016.1242497

Published online: 01 Dec 2016.

SOFT MATERIALS
http://dx.doi.org/10.1080/1539445X.2016.1242497

Taylor & Francis
Taylor & Francis Group

Depth-dependent mechanical characteristics of Porcine cornea

Ruiqi Du[a], Hanjing Tian[a], Xianghong Xu[a,b], Yingfeng Shao[a,b], and Fan Song[a,b]

[a]State Key Laboratory of Nonlinear Mechanics (LNM), Institute of Mechanics, Chinese Academy of Sciences, Beijing, China; [b]Beijing Key Laboratory of Engineered Construction and Mechanobiology, Institute of Mechanics, Chinese Academy of Sciences, Beijing, China

ABSTRACT
The biomechanical characteristics of porcine corneal stroma along the depth of cornea in different anatomical orientations were experimentally investigated and quantitatively analyzed. The porcine corneal stroma tested here was cut mechanically into the three layers along the thickness of the cornea for the first time. Based on the convenient uniaxial tensile extensometry, the Young's moduli of each stromal layer in different anatomical orientations were proved to be equal to each other. By virtue of testing the mechanical behaviors of the different layers, the Young's moduli of the stromal layers were found to decrease gradually from the anterior to posterior corneal surface. Thus, the stroma was proved to be mechanically transversely isotropic. Furthermore, based on the analysis of the experimental data, the change of stromal Young's modulus along the depth of the cornea was determined to be linear and equal to - 2.79 ± 0.967 MPa/mm from the anterior to posterior corneal surface. Finally, the linear gradient characteristic of the stroma was appropriately explained by virtue of stromal microstructures.

ARTICLE HISTORY
Received 12 April 2016
Accepted 23 September 2016

KEYWORDS
Anisotropy; corneal stroma; linear gradient; mechanical properties; uniaxial tension experiment

Introduction

The biomechanical properties of the cornea play a key role in maintaining the normal physiological functions of the eye, and their changes are associated intimately with various kinds of corneal diseases in clinical diagnosis and treatment. Corneal biomechanical properties matter a lot in the process of obtaining the accurate measurement value of intraocular pressure, which is the only controllable variable to increase the possibility of detection and to slow down the progression of glaucoma (1, 2). Corneal collagen cross-linking is widely used to treat the keratoconus and corneal ectasia after the laser *in-situ* keratomileusis, the main purpose of which is to increase the corneal mechanical rigidity (3). In addition, the precise measurement of corneal biomechanical properties is also a vital factor in avoiding compliance mismatch in the operation of the keratoprosthesis, corneal onlay, and corneal inlay (4).

Corneal mechanical behaviors, as well as structural integrity, stem intrinsically from the corneal stroma, which constitutes approximately 90% of the thickness of the whole cornea (5). However, the mechanical properties of corneal stroma have not been understood very well yet. To date, there are no conclusive and consistent data on the magnitude of stromal biomechanical properties. For example, the values of stromal Young's modulus reported in the literature vary widely, ranging roughly from 0.01 to 10 MPa (6–8). With the development of new therapy methods for corneal disease, more comprehensive insights into corneal mechanical properties, such as the biomechanical properties of stroma along the thickness of cornea, now become one of the focuses in ophthalmological research.

There have been many studies on the corneal mechanical properties through stromal thickness using different research methods so far (9–13). Kohlhass et al. cut the corneal stroma located in the upper 400 microns into two layers and tested their mechanical properties using uniaxial tensile extensometry (9). Winkler et al. and Dias and Ziebarth also cut the corneal stroma into two layers and examined their mechanical properties using Atomic Force Microscope (10, 11). Scarcelli et al. investigated the difference of Young's modulus between the anterior and posterior corneal stroma using a Brillouin microscope (12). All these studies above found that there was a significant difference between the mechanical behaviors of the anterior and posterior corneal stroma, i.e. the anterior was stiffer (9–12) and there was a gradient change of elasticity within the corneal stroma (11). In addition, Randlemann et al. examined the cohesive tensile strength at the different depths in the corneal stroma and

CONTACT Fan Song songf@lnm.imech.ac.cn State Key Laboratory of Nonlinear Mechanics (LNM) and Beijing Key Laboratory of Engineered Construction and Mechanobiology, Institute of Mechanics, Chinese Academy of Sciences, Beisihuanxi Road No.15, Beijing, 100190, China.
Color versions of one or more of the figures in the article can be found online at www.tandfonline.com/lsfm.

pointed out that anterior stroma had a larger adhesion force than the posterior one (13). Obviously, in the existing studies on the depth-dependent behaviors, all corneal stromal specimens tested were almost divided only into the two layers, the anterior and the posterior. However, to obtain the mechanical properties of the stroma along the thickness of the cornea, specially, such as gradient characteristics, it is not enough to only test and compare the mechanical behaviors of two stromal layers. The biomechanical behaviors of three or more stromal layers need to be examined. In addition, the variations of the mechanical behaviors of corneal stroma in different anatomical orientations throughout the thickness, which is important in practice, have not been understood very well yet so far.

In the existing experimental studies, porcine cornea was widely used as a substitute for scarce normal human donor cornea. It is because porcine cornea is, first, easily available; and second, numerous comparative investigations prove that the tensile strength and the short-term nonlinear stress–strain behaviors between porcine and human cornea are very similar, which results in porcine cornea becoming a good research model of human cornea (14, 15).

In the present study, porcine corneal stroma was first cut into three layers along the thickness of the cornea, i.e. anterior, central, and posterior layers. Moreover, the biomechanical behaviors of the stroma in the three anatomical orientations, the vertical, horizontal, and diagonal, were systematically tested and quantitatively analyzed. The study here aims at: (*i*) examining the mechanical anisotropic characteristics of corneal stroma and (*ii*) determining the variation of the mechanical behaviors along the stromal depth in the three anatomical orientations. Thus, based on the experiments of the mechanical behaviors, the Young's moduli in all the orientations were found to be equal to each other in the same layer of stroma, i.e. the cornea was mechanically transversely isotropic. By testing the mechanical behaviors of the different layers, we obtained that the Young's modulus of the stroma gradually decreased from the anterior to posterior corneal surface. Further analysis proved that the elastic gradient of the stroma was linear in its thickness, and this gradient value was determined to be - 2.79 ± 0.967 MPa/mm from the anterior to the posterior. Finally, the mechanical behaviors of the stroma were appropriately explained by virtue of its microstructures.

Materials and methods

Materials

All test specimens were derived from the fresh eyes of the pigs aged from 4 to 6 months, from a local slaughterhouse within 3 h postmortem. Once the porcine eyes were delivered to the laboratory, the corneal epithelium was removed with a cotton-tipped applicator. Then the whole eyeballs without the corneal epithelium were submerged in 20% Dextran solution (20 g of Dextran in 100 mL of PBS; avg. molecular weight: 70,000 g/mol, Pharmacia, Piscataway, NJ), with the cornea side down, to reinstate the corneal thickness to the physiological level (16). Note that these specimens were submerged in 20% Dextran solution for at most 4 h in a refrigerator at 4°C. After being stored for 2 h, they were taken out one by one and prepared for the experiments. The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Institute of Mechanics of the Chinese Academy of Sciences.

Two incisions were performed on the surface of each cornea 2 mm away from the corneal limbus with a diamond knife with a double footplate and an adjustable blade (KOI, San Diego, CA). The depths of the incisions were 300 and 600 microns, respectively. In the direction vertical to the incisions, the stroma was dissected separately into anterior, central, and posterior layers with a surgical knife (Crescent Knife, bevel up; Sharpoint, Reading, PA). Then a corneal button with a 3–4 mm scleral ring was removed from each eyeball using a pair of scissors. The iris, lens, and ciliary body were all removed, too. A corneal strip with 3 mm width was obtained from the central part of the cornea using a double-blade tool. The strips were anatomically orientated to be the superior-inferior (vertical), temporal-nasal (horizontal), and 45° and 135° diagonal directions, respectively. It is easy to determine these directions owing to the distinct difference between the corneal vertical and horizontal diameters. However, with the side of the corneas unknown, diagonal specimens were taken from either the 45° or 135° direction according to the symmetry of the cornea (17).

The length of the corneal strip specimens ranged from 16 to 20 mm, in which the gauge length was 10 mm. The thickness of each specimen was measured using an ultrasound pachymeter (PachPen, Accutome, Malvern, PA) and a micrometer (Mitutoyo, Tokyo). The mean value ± standard deviation (SD) of the whole corneal thickness was measured to be 979 ± 37 microns, and the mean values ± SDs of the specimen thickness of the anterior, central, and posterior layers were measured to be 313 ± 17, 324 ± 19, and 342 ± 30 microns, respectively.

Test methods

We employed conventional uniaxial tensile testing to investigate the mechanical properties of the corneal stroma. The total number of the specimen tested at each anatomical orientation (vertical, horizontal, and diagonal) was 72, in which the number of the specimen of one layer (anterior, central, or posterior) was 24. The experiments were carried out on an Instron 5848 materials testing machine (Instron, Norwood, MA) equipped with a 5 N capacity load cell at room temperature (22°C). The specimens of the corneal stroma were attached to the mechanical clamps with 320-grit sandpaper to avoid slippage. An ultrasound moistener was used to keep the specimen moist. The output including specimen elongation in millimeter and the axial tension load in Newton were collected automatically by the computer. These values were recorded per 0.05 s for further analysis.

At the beginning of the experiments, the specimens were loaded and unloaded in the range from 0.01 to 0.1 of the original length under a constant velocity of loading (3 mm/min) for five cycles to be preconditioned for stabilizing their behaviors. Then, to reproduce the physiological stress state of the cornea, an initial axial load of 50 mN was applied before starting the monotonic stretching of the specimens. The specimens were then loaded up to failure under a constant velocity of stretching (1 mm/min) just 300 s after their recovery.

Data analysis

A uniaxial stress–strain curve of the corneal stroma is usually nonlinear and the true Young's modulus usually varies over a wide range. Therefore, here we used Fung's method (18) to obtain the Young's modulus of the stroma, which had been widely employed in the study of biomaterial mechanical properties (6). A usual exponential stress–strain relationship

$$\sigma + C = Ae^{B\varepsilon} \tag{1}$$

was employed to closely fit the experimental curves under small stress ($\sigma < 0.1$ MPa). Therefore, the values of the coefficients A, B, and C in Eq. (1) were determined. Differentiating the stress, σ, with respect to the strain, ε, determined the expression of Young's modulus:

$$E = \frac{d\sigma}{d\varepsilon} = ABe^{B\varepsilon} = B(\sigma + C) \tag{2}$$

Accordingly, the Young's modulus related to a stress value was obtained by Eq. (2).

Statistical analysis

A one-way ANOVA test was used to assess the anisotropy in the three anatomical directions and to analyze the difference among the behaviors of the anterior, central, and posterior layers. Analysis of all data was performed using the Statistical Package (SPSS, version 19.0, IBM Corporation), and all statistical tests were carried out with a significant level of risk set at α = 5%. All data are presented as the mean value ± SD.

Results and discussion

The different stromal layers derived from the same cornea were carried out in the tests elongating up to failure in the diagonal orientation. The stress–strain relationships of the three layers were all nonlinear, as shown in Fig. 1. The maximum stresses of the anterior, central, and posterior layers were approximately measured to be 5.43, 2.72, and 2.02 MPa, respectively, and the maximum strain of all the specimens was less than

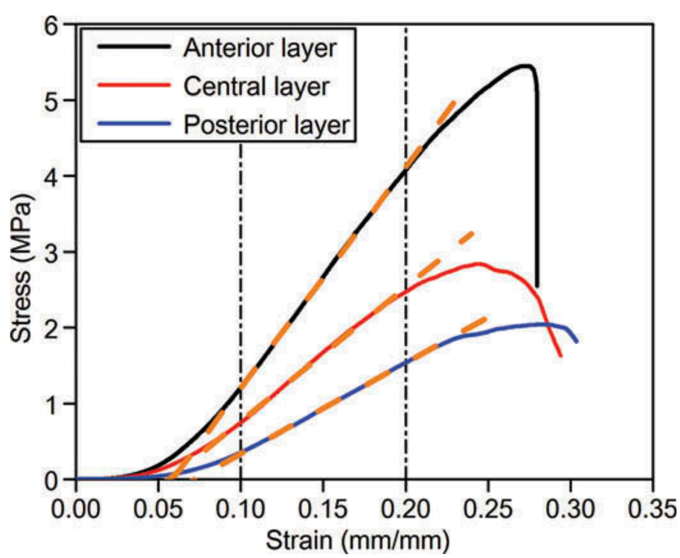

Figure 1. Uniaxial stress-strain curves that specimen is stretched up to failure. This graph showed the tensile behaviors of the different stroma layers taken from the same cornea.

0.30. Specifically, all the tensile stress–strain curves of the three layers could be roughly divided into three parts in the direction of the strain. When the strain was less than 0.1, the relationship of stress–strain was nonlinear, where the region where the strain was less than 0.02 was considered as the relaxed length of the specimen (18, 19); when the strain was between 0.1 and 0.2, the relationship of stress–strain approximated to be linear, the fitting degrees of linearity approximated 0.9997, 0.9982, and 0.9997 for the anterior, central, and posterior layers, respectively, and the Young's moduli of the three layers were computed to be 29.05, 17.66, and 11.99 MPa, respectively; and when the strain was greater than 0.2, the nonlinear relationship of stress–strain reappeared. The region where the strain is greater than 0.1, in fact, is outside of the range of corneal deformation in the normal physiological state of human.

We focused on the nonlinear mechanical behaviors of all stromal layers along the different anatomical orientations under the small strain of cornea ($\varepsilon < 0.12$). First, the mechanical behavior of each layer (the anterior, central, and posterior) in the three anatomical orientations was experimentally proved to be equal, as shown in Fig. 2. In the anterior layer, the maximum error among the stresses of the three anatomical orientations under the same strain was measured to be less than 0.2 MPa; in the central layer, the maximum error was less than 0.1 MPa; and in the posterior layer, the maximum error decreased to less than 0.06 MPa. Correspondingly, the maximum error of all the Young's moduli of the three stromal layers was calculated to be less than 23%. In order to make the statistical analysis on the errors of Young's modulus, for example, the Young's modulus was calculated at different stromal layers in different anatomical orientations at the stress of 0.03MPa and 0.04MPa, respectively; the mean value and

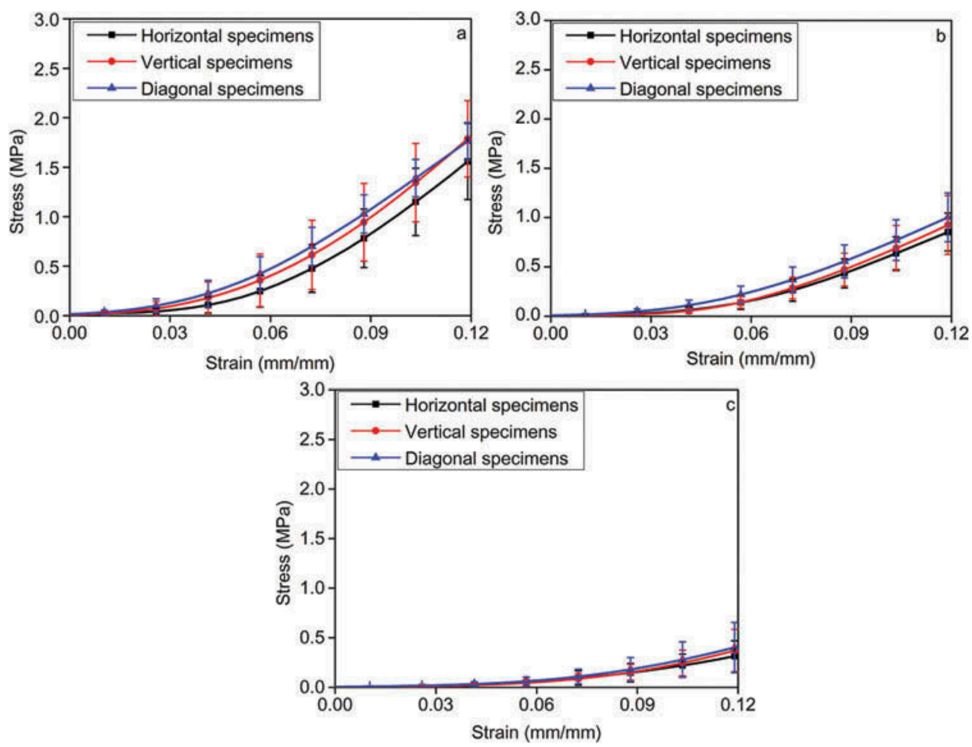

Figure 2. Average uniaxial stress-strain curves of the specimens taken from the corneal stroma at the same stromal depth in different anatomical orientations. (a), (b) and (c) showed the average uniaxial stress-strain curves of the specimens taken from the anterior, central and the posterior, respectively.

Table 1. Young's modulus, *E*, of corneal strip specimens (data shown are MPa).

		Vertical direction		Horizontal direction		Diagonal direction		Overall directions	
	Stress	Mean *E*	SD	Mean *E*	SD	Mean *E*	SD	Mean *E*	SD
Anterior	0.03	2.869	0.584 (20.3%)	2.484	0.740 (29.8%)	2.706	0.707 (26.1%)	2.687	0.670 (22.9%)
	0.04	3.681	0.616 (16.7%)	3.259	0.855 (26.2%)	3.404	0.712 (20.9%)	3.448	0.724 (21.0%)
Central	0.03	2.333	0.337 (14.5%)	2.098	0.536 (25.5%)	2.071	0.584 (28.2%)	2.167	0.490 (22.6%)
	0.04	3.088	0.406 (13.1%)	2.787	0.556 (19.9%)	2.656	0.696 (26.2%)	2.844	0.571 (20.1%)
Posterior	0.03	1.640	0.331 (20.2%)	1.746	0.386 (22.1%)	1.415	0.228 (16.1%)	1.600	0.338 (21.1%)
	0.04	2.205	0.351 (15.9%)	2.289	0.482 (21.1%)	1.890	0.270 (14.3%)	2.128	0.402 (18.9%)

SD are listed in Table 1. A one-way ANOVA test was used to compare the Young's modulus differences among the three anatomical orientations, and the results showed statistically insignificant differences ($p > 0.05$). This was an indication that the mechanical difference among the different anatomical orientations within the same stromal layers could be deemed to be very small, i.e. the mechanical behavior within each stromal layer in the different anatomical orientations was isotropic. In addition, the mechanical homogeneity of the corneal stroma increased from the anterior to posterior, as shown in Fig. 2.

Second, in each anatomical orientation, there were clear differences in the mechanical behaviors of the anterior, central, and posterior stromal layers, and all the Young's moduli of the stroma decrease gradually from the anterior to posterior corneal layers, as shown in Fig. 3. It was implied that there was an elastic gradient of Young's modulus through the thickness direction of the corneal stroma.

In order to determine quantitatively the mechanical gradient characteristics of the cornea, without loss of generality, the change in Young's moduli at a series of stress values was investigated in detail. Based on Eqs. (1) and (2), we readily obtained the average Young's moduli at the average depth of each corneal stromal layer under the stresses ranging from 0.02 MPa to 0.09 MPa, for example, as listed in Table 1 at the stresses of 0.03 and 0.04 MPa, and then connected the average Young's moduli at each stress value by virtue of fitting a linear regression line, as shown in Fig. 4. The degrees of fitting were computed to range from 0.9797 to 0.9994, which was of excellent linear fits. The results here proved that along the thickness of the corneal stroma, all the Young's moduli of the stroma displayed a linear gradient at each given stress. Furthermore, the average slope and SD of the lines was determined to be - 2.79 ± 0.967 MPa/mm. This was an indication that all the lines of the Young's moduli varying with the thickness of the cornea could be considered to be parallel to one another. It was proved that the change in Young's modulus of the stroma along the corneal thickness was roughly consistent. Namely, the Young's modulus of the stroma linearly and continuously decreased from the anterior to posterior corneal surface. The average gradient of the Young's modulus in the depth direction of the cornea was - 2.79 MPa/mm.

The mechanical behaviors of the corneal stroma stem intrinsically from the microstructure of the stroma (5, 20). The microstructure of the stroma is made up of 200–400 superimposed lamellae of fine collagen fibrils regularly spaced and embedded within an extracellular matrix (21). An X-ray diffraction study on the microstructure demonstrated that the collagen fiber structure had mainly circumferential orientation and showed no preferential orientation in any meridian

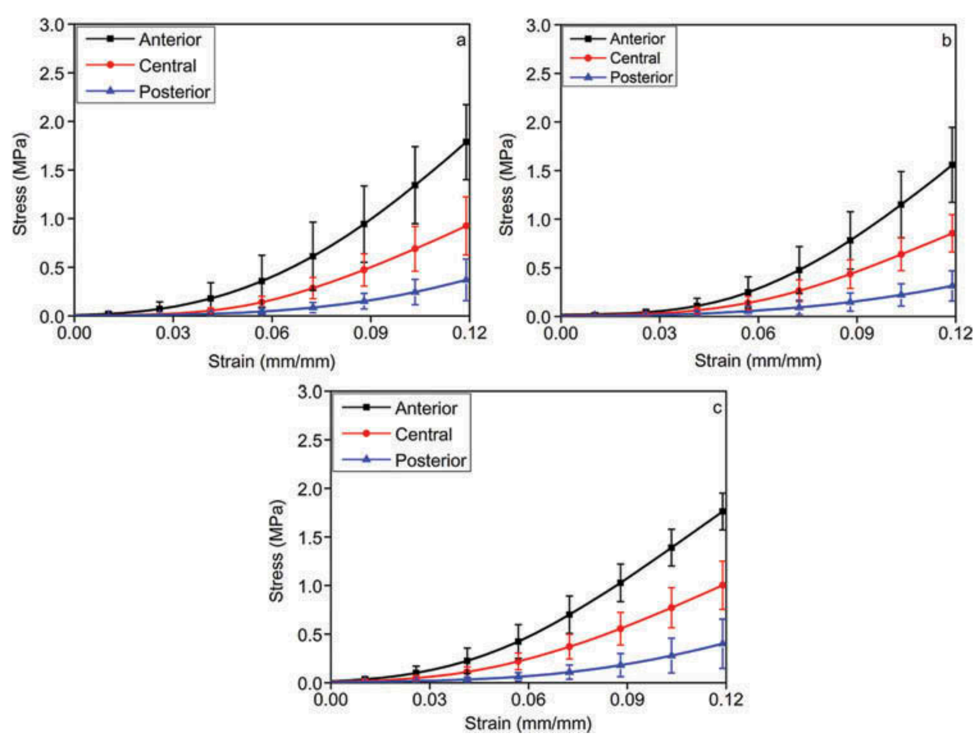

Figure 3. Average uniaxial stress-strain curves of the specimens of corneal stroma in the same orientation at different stromal depths. (a), (b) and (c) showed the average uniaxial stress-strain of specimens taken from the vertical, horizontal and diagonal orientations, respectively.

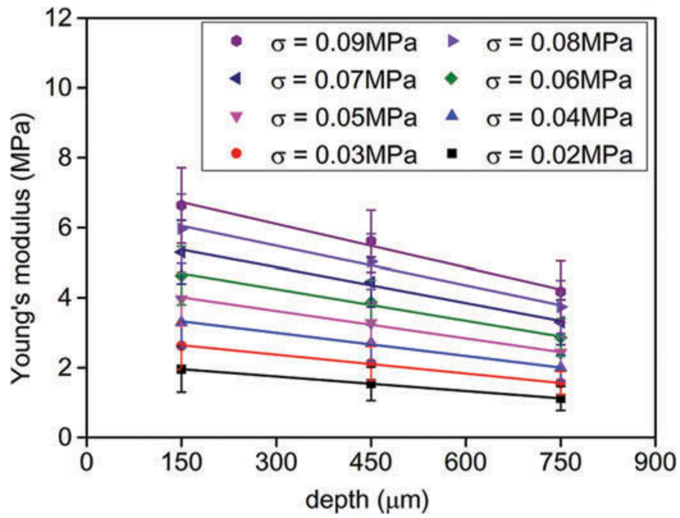

Figure 4. The fit lines of the Young's moduli along stromal thickness at different stress values. These lines were roughly parallel to one another and showed the consistency of the linear gradient of stroma.

direction (22). Obviously, this microstructure just corresponded to the mechanical isotropy of the corneal stroma in the direction vertical to the thickness of the cornea. Some investigations indicated that there were regional differences in the collagen lamellar orientation throughout the thickness of the stroma (23, 24). The collagen fibrils mostly array parallel to the anterior surface within the lamellae, but the interweaving of lamellae remarkably increases from the posterior toward the anterior surface (5, 24–26). It can be explained from the microstructure of the corneal stroma that the increased lamella interweaving in the stroma forms the mechanical gradient of the stroma along the thickness of the cornea. In addition, from the viewpoint of physiological function, the anterior stromal layer with stiffer and isotropic biomechanical properties is helpful to maintain the same curvature of the anterior surface under the variation of intraocular pressure and hydration state of the cornea (27).

Conclusions

The depth-dependent mechanical behaviors of the corneal stroma were experimentally investigated and quantitatively analyzed. The results in the present study display that (*i*) within each stromal layer perpendicular to the thickness direction of the cornea, the Young's moduli in the different anatomical orientations are equal; (*ii*) in the thickness direction of the cornea, the mechanical behaviors of the different layers are apparently different and gradually decrease from the anterior to the posterior. Thus, the cornea is mechanically transversely isotropic; and (*iii*) the Young's modulus of the stroma formed a linear gradient along the thickness of the cornea.

Acknowledgments

The authors are grateful to Yihui Feng, Ph.D., ke Chen, MS, and Zhiqiang Shen, MS, for their help during the materials testing.

Funding

The authors wish to thank the National Natural Science Foundations of China (grant numbers 11232013 and 11472285).

References

1. Liu, J., and Roberts, C.J. (2005) Influence of corneal biomechanical properties on intraocular pressure measurement: quantitative analysis. *Journal of Cataract & Refractive Surgery*, 31(1):146–155.
2. Quigley, H.A. (2011) Glaucoma. *Lancet*, 377 (9774):1367–1377.
3. Snibson, G.R. (2010) Collagen cross-linking: a new treatment paradigm in corneal disease - a review. *Clinical and Experimental Ophthalmology*, 38(2):141–153.
4. Ruberti, J.W., Roy, A.S., and Roberts, C.J. (2011) Corneal biomechanics and biomaterials. *Annual Review of Biomedical Engineering*, 13:269–295.
5. DelMonte, D.W., and Kim, T. (2011) Anatomy and physiology of the cornea. *Journal of Cataract & Refractive Surgery*, 37(3):588–598.
6. Hoeltzel, D.A., Altman, P., Buzard, K., and Choe, K.-i. (1992) Strip extensiometry for comparison of the mechanical response of bovine, rabbit, and human corneas. *Journal of Biomechanical Engineering*, 114 (2):202–215.
7. Jue, B., and Maurice, D.M. (1986) The mechanical properties of the rabbit and human cornea. *Journal of Biomechanics*, 19(10):847–853.
8. Schwartz, N., Mackay, R.S., and Sackman, J. (1966) A theoretical and experimental study of the mechanical behavior of the cornea with application to the

measurement of intraocular pressure. *The Bulletin of Mathematical Biophysics*, 28(4):585–643.
9. Kohlhaas. M., Spoerl, E., Schilde, T., Unger, G., Wittig, C., and Pillunat, L.E. (2006) Biomechanical evidence of the distribution of cross-links in corneas treated with riboflavin and ultraviolet A light. *Journal of Cataract & Refractive Surgery*, 32(2):279–283.
10. Winkler, M., Chai, D., Kriling, S., Nien, C.J., Brown, D. J., Jester, B., Juhasz, T., and Jester, J.V. (2011) Nonlinear optical macroscopic assessment of 3-D corneal collagen organization and axial biomechanics. *Investigative Ophthalmology & Visual Science*, 52(12):8818.
11. Dias, J.M., and Ziebarth, N.M. (2013) Anterior and posterior corneal stroma elasticity assessed using nanoindentation. *Experimental Eye Research*, 115:41–46.
12. Scarcelli, G., Kling, S., Quijano, E., Pineda, R., Marcos, S., and Yun, S.H. (2013) Brillouin microscopy of collagen crosslinking: noncontact depth-dependent analysis of corneal elastic modulus brillouin microscopy of collagen crosslinking. *Investigative Ophthalmology & Visual Science*, 54(2):1418–1425.
13. Randleman, J., Dawson, D., Grossniklaus, H., McCarey, B., and Edelhauser, H. (2008) Depth-dependent cohesive tensile strength in human donor corneas: implications for refractive surgery. *Journal of Refractive Surgery* (Thorofare, NJ: 1995), 24(1):S85.
14. Elsheikh, A., Alhasso, D., and Rama, P. (2008) Biomechanical properties of human and porcine corneas. *Experimental Eye Research*, 86(5):783–790.
15. Zeng, Y., Yang, J., Huang, K., Lee, Z., and Lee, X. (2001) A comparison of biomechanical properties between human and porcine cornea. *Journal of Biomechanics*, 34 (4):533–537.
16. Borja, D., Manns, F., Lamar, P., Rosen, A., Fernandez. V., and Parel, J.-M. (2004) Preparation and hydration control of corneal tissue strips for experimental use. *Cornea*, 23(1):61–66.
17. Elsheikh, A., and Alhasso, D. (2009) Mechanical anisotropy of porcine cornea and correlation with stromal microstructure. *Experimental Eye Research*, 88(6):1084–1091.
18. Fung, Y. (1967) Elasticity of soft tissues in simple elongation. *American Journal of Physiology–Legacy Content*, 213(6):1532–1544.
19. Boschetti, F., Triacca, V., Spinelli, L., and Pandolfi, A. (2012) Mechanical characterization of porcine corneas. *Journal of Biomechanical Engineering-Transactions of the Asme*, 134(3):451–459.
20. Meyers, M.A., Chen, P.-Y., Lin, A.Y.-M., and Seki, Y. (2008) Biological materials: structure and mechanical properties. *Progress in Materials Science*, 53(1):1–206.
21. Bergmanson, J.P., Horne, J., Doughty, M.J., Garcia, M., and Gondo, M. (2005) Assessment of the number of lamellae in the central region of the normal human corneal stroma at the resolution of the transmission electron microscope. *Eye & Contact Lens*, 31 (6):281–287.
22. Hayes, S., Boote, C., Lewis, J., Sheppard, J., Abahussin, M., Quantock, A.J., Purslow, C., Votruba, M., and Meek, K.M. (2007) Comparative study of fibrillar collagen arrangement in the corneas of primates and other mammals. *The Anatomical Record*, 290 (12):1542–1550.
23. Kamma-Lorger, C.S., Boote, C., Hayes, S., Moger, J., Burghammer, M., Knupp, C., Quantock, A.J., Sorensen, T., Di Cola, E., and White, N. (2010) Collagen and mature elastic fibre organisation as a function of depth in the human cornea and limbus. *Journal of Structural Biology*, 169(3):424–430.
24. Abahussin, M.O., Hayes, S., Knox Cartwright, N.E., Kamma-Lorger, C.S., Khan, Y., Marshall, J., and Meek, K.M.A. (2009) 3D collagen orientation study of the human cornea using X-ray diffraction and femtosecond laser technology. *Investigative Ophthalmology & Visual Science*, 50(11):5159–5164.
25. Bron, A. (2001) The architecture of the corneal stroma. *British Journal of Ophthalmology*, 85(4):379–381.
26. Komai, Y., and Ushiki, T. (1991) The three-dimensional organization of collagen fibrils in the human cornea and sclera. *Investigative Ophthalmology & Visual Science*, 32(8):2244–2258.
27. Müller, L.J., Pels, E., and Vrensen, G.F. (2001) The specific architecture of the anterior stroma accounts for maintenance of corneal curvature. *British Journal of Ophthalmology*, 85(4):437–443.