A NOVEL INDENTATION TECHNIQUE FOR THE MECHANICAL CHARACTERIZATION OF HUMAN CORNEA

* M. Ahearne, * Y. Yang, * A.J. El Haj, ** K.Y. Then and * K.K. Liu

* Institute of Science and Technology in Medicine, Keele University, Stoke-on-Trent, United Kingdom
** Birmingham and Midland Eye Centre, City Hospital, Birmingham, United Kingdom

i.k.liu@keele.ac.uk

Abstract: Cornea is a load bearing tissue whose mechanical characteristics are poorly understood. At present there are no suitable methods to examine the mechanical properties of corneas. A novel method of examining the mechanical and viscoelastic characteristics of corneas has been developed. A test rig consisting of an indenter with a spherical tip connected to force transducer has been set up. The force transducer was connected to a motorised arm that allowed the indenter to be lowered vertically onto the centre of a cornea, which was clamped around its outer circumference. The indenter is then further lowered to apply a load to the centre of the cornea. The degree of central displacement and the force recorded by the transducer were used to obtain hysteresis and stress relaxation curves for human corneas. A theoretical model has been developed to describe the loading and unloading curves during indentation. Our results show that this system can be used to characterise the mechanical and viscoelastic properties of human corneas.

Introduction

The cornea is a multi-layered, anisotropic, viscoelastic, load bearing tissue whose primary functions are to focus light as it enters the eye and protect the eye from debris. The mechanical properties of the cornea are important in maintaining these functions. Force is applied to the cornea from intraocullar pressure, atmospheric pressure and movement of the eyelid. Disease, injury and surgery are believed to have significant impact on the mechanical properties of the cornea. A better understanding of these properties is of importance in the development of corneal surgical procedures, particularly with the increase in the number of refractive surgical procedures in recent years. The mechanical properties of corneas are also of importance in the development of alternatives to corneal tissue transplants such as keratoprosthesis and tissue engineered corneas.

At present the two most commonly used methods to determine the mechanical properties of corneal tissue are strip extensiometry and bulge testing. Strip extensiometry [1-3] has been extensively used to study the mechanical behaviour of corneal tissue. This method involves applying a tensile force to strips of cornea held between two grips. There are a number of shortcomings with using this method, which may generate inaccuracy in the determination of the cornea's mechanical properties. These include the potential misalignment of grips, the curvature of the cornea not being taken into consideration and the tension being primarily on fibrils that are parallel to the direction of strain [4]. Recent attempts at overcoming some of these shortcomings though the application by mathematical modelling have shown some success but this also introduces new problems and involves highly complicated calculations [5]. Bulge testing [5,6] involves inflating the cornea through a window in the substrate and measuring the resulting strain. This method also has a number of shortcomings including potential leakage, difficulty to control applied pressure and dissolved air becoming trapped in the solution. An alternative approach, which is free from aforementioned intractability, is highly desirable. We have developed a point loading indentation system to examine the mechanical properties of whole human corneas, which is based on a system used to measure the mechanical properties of thin biological membranes [7].

Materials and Methods

Human corneas were obtained from the corneal transplant service eye bank (Bristol and Manchester, UK). The corneas used were deemed unsuitable for transplantation due to low endothelial cell numbers. All the experiments were carried out within 2 weeks of receiving corneas. The thickness of the corneas was determined using optical coherence tomography (OCT). OCT is a non-invasive imaging technique capable of micrometer resolution and has previously been used to measure the thickness of corneas in vivo [8,9]. It works by passing a laser beam through the cornea and then measuring the intensity and echo time delay of the backscattered beam. This information can be used to create an image of the corneas cross-section. The optical density of the cornea can be used to calibrate the image thickness [9]. A micrometer was also used to verify corneas thickness.

Prior to indentation, the corneas had to be clamped around their outer circumference using a specially designed sample holder. This holder consists of two circular rubber rings attached to two thin steel plates with holes in the plates that align with the hole in rings. The cornea is placed between the rings, which are clamped together by a number of screws. Figure 1 shows a cornea that is being held in the sample holder. The shape of the rings allows the cornea to be clamped firmly without any unnatural bending. An image of the curvature of the cornea, while it was in the sample holder, was recorded using a previously described image acquisition system [10] consisting of a long distance microscope connected to a CCD camera. A spray containing Dulbecco's modified eagle medium (Biosera, France) and antibiotic and antimitotic solution (Sigma, UK) was used to prevent dehydration of the cornea while clamped. A small water try was placed under the sample holder to increase the local humidity.

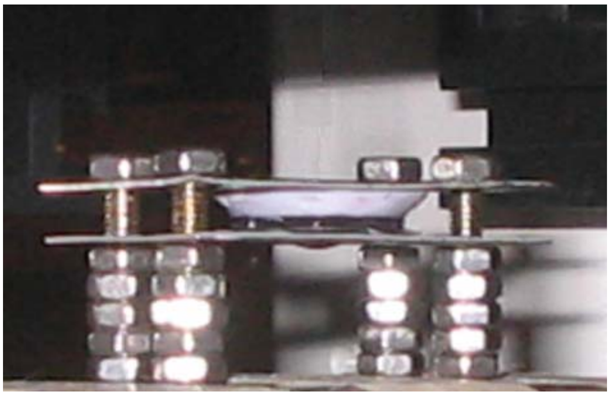

Figure 1: Photographic image of a cornea clamped firmly in a sample holder

A schematic diagram of the indentation system used to examine the corneas mechanical and viscoelastic characteristics is shown in figure 2. It consists of a force transducer (404A, Aurora Scientific Inc., Canada) connected to a thin glass cylindrical indenter with a hemispherical tip. The tip has a radius of 190 µm, which was measured using the image acquisition system. The force transducer is attached to an arm, which is attached to a motorised z-axial linear displacement actuator (Cell Robotics Inc., USA). This indentation system has a force resolution of 2 µN and is capable of displacement steps as small as 100 nm. The sample holder was placed onto a X-Y translation stage (Cell Robotics Inc., USA) under the indenter. The stage was moved so that the indenter aligned vertically with the centre of the cornea. The indenter was then lowered until the tip met the surface of the cornea. This was considered to be the start point for indentation. The indenter was then lowered a pre-determined distance causing the cornea to deform. The resulting force transducer signal was filtered and amplified using differential and buffer amplifiers (S 404A, Aurora Scientific Inc., Canada). The amplified analogue signal was transmitted through a connector block (SCB-68, National Instruments, USA) to a data acquisition (DAQ) board (NI PCI-6036E, National Instruments, USA) for digitisation and further processing. LabView (National Instruments, USA) was used to create a programme capable of acquiring and processing the data. Calibration of the instrument was performed by placing objects of known weight directly onto the top of the tip.

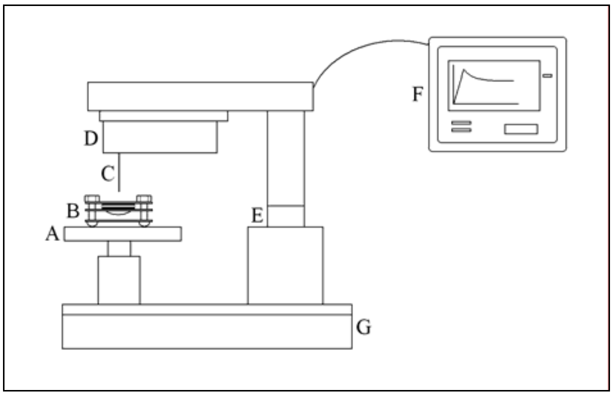

Figure 2: Schematic representation of instrument: (A) x-y translation stage; (B) cornea and sample holder; (C) hemispherical tipped indenter; (D) force transducer; (E) z-axial displacement actuator; (F) data processing and display CPU; (G) anti-vibration table

A simple theoretical model was developed to describe the mechanics of the cornea deformation during loading and unloading processes. A diagram depicting the theoretical model of an indented cornea is shown in figure 3. In order to determine the relationship between the applied force and the strain it is assumed that the cornea is isotropic and conical in shape.

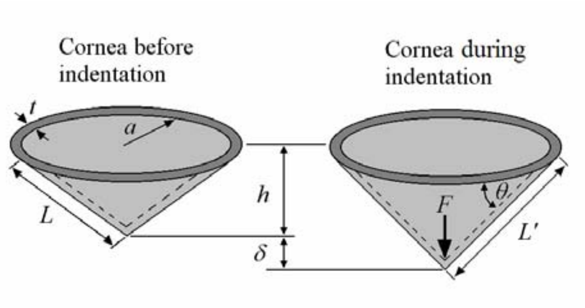

Figure 3: Theoretical model of cornea before and during indentation

At an indentation depth of δ, the strain, ε, can be assumed to be,

$$\varepsilon = \frac{L' - L}{L} \qquad (1)$$

where L is the distance from the clamped edge of the cornea to the centre of the cornea before indentation and L' is the distance from the clamped edge of the cornea to the centre of the cornea during indentation. The values L and L' can be easily determined from the equations,

ISSN: 1727-1983 © 2005 IFMBE

$$L = \sqrt{a^2 + h^2} \quad (2)$$

$$L' = \sqrt{a^2 + (h+\delta)^2} \quad (3)$$

where h is the vertical height of the clamped cornea and a is the radius at the clamped portion of the cornea. The force applied to the cornea by the indenter can be considered to be equivalent to a point load as the indenter radius is significantly smaller than the radius of the clamped portion. The force, F, applied to the cornea can be described as,

$$F = \sigma.2\pi ta Sin\theta \quad (4)$$

where σ is the resulting stress around the clamped portion of the cornea, θ is the angle between the cornea edge and the horizontal and t is the cornea thickness. $Sin\ \theta$ can be determined from the equation,

$$Sin\theta = \frac{h+\delta}{L'} \quad (5)$$

It is generally accepted that most biological materials including cornea exhibit a non-linear viscoelastic relationship between stress and strain and therefore a single value for the elastic modulus cannot be accurately obtained [11]. Hoeltzel et al. [2] used the equation,

$$\sigma = \alpha(\varepsilon - \varepsilon_s)^\beta \quad (6)$$

to define the relationship between tensile stress and strain where α represents a scaling factor, β represents the non-linear component between stress and strain and ε_S is the slack strain (the lowest strain to result in an increase in stress). By combing equations 4 and 6, the relationship between force and strain can be described by the equation,

$$F = \alpha(\varepsilon - \varepsilon_s)^\beta 2\pi ta Sin\theta \quad (7)$$

Non-linear regression analysis was performed using XLStat (Addinsoft, France) to determine the values for α and β that best represent the loading and unloading curves caused by indentation. These values were then substituted back into equation 7 to examine the correlation between the actual data and the theoretical data.

Results

A typical OCT image of the cross-section of the central portion of a human cornea is shown in figure 4. The thickness of the cornea can easily be determined from the image. The corneas examined for this paper were found to have thickness approaching 1 mm, which is slightly higher than found in-vivo in healthy people. This would suggest that some swelling occurred while the cornea was stored in media. Other reports have also found a similar swelling effect after human corneas were extracted from donor eyes [2,3]. Measurements taken using a micrometer confirmed OCT thickness measurements.

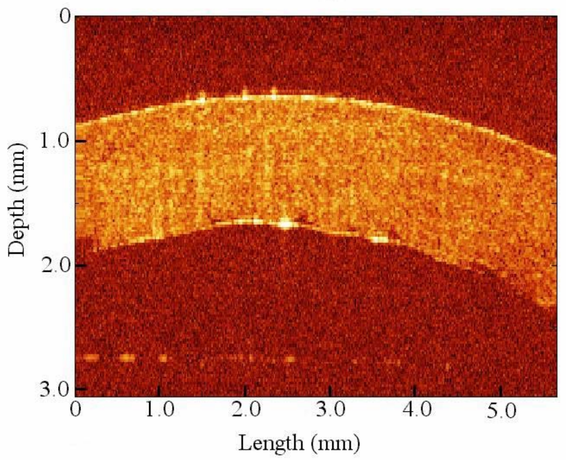

Figure 4: OCT image of the cross-section of the central portion of a human cornea

The results from typical force-displacement curves for both actual data and theoretical data are shown in figure 5. It can be seen that there was a high degree of correlation between the two curves. This was true for all the data collected by this method. The coefficients of determination (R^2) were found to range from 0.996 to 1.000, which is almost a perfect fit for the corneas tested. The values for α and β are similar but slightly lower than values obtained using extensiometry on strips of corneal tissue in a previous studies [2-3]. The different loading mechanism used for the extensiometry and the potential damage caused to the structure of the corneal tissue while being cut into strips could explain the difference between our results and those reported in [2-3]. As expected, the values for both α and β also differ during the loading and unloading cycles.

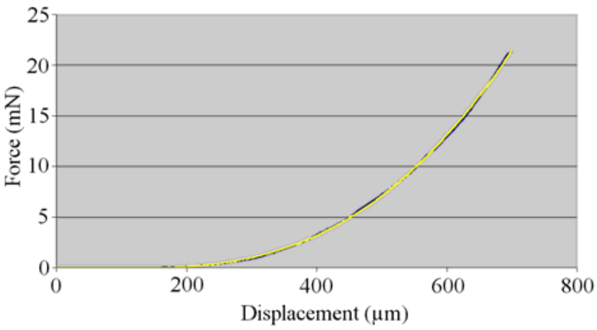

Figure 5: Experimental (black) and theoretical (yellow) loading curves for a cornea during indentation

The load-unloading curves for a typical cornea indented to 700 µm 5 times are displayed in figure 6. As expected the loading and unloading curves do not match due to hysteresis, a common feature among viscoelastic biological tissues [12]. It can also be seen that the amount of force required to indent the cornea decreased with each loading-unloading cycle. This is due to internal restructuring of the cornea after each cycle. This type of restructuring occurs to minimise the effect of the force on the tissue. It can be seen that there is little change in the force-displacement curve for the final two cycles.

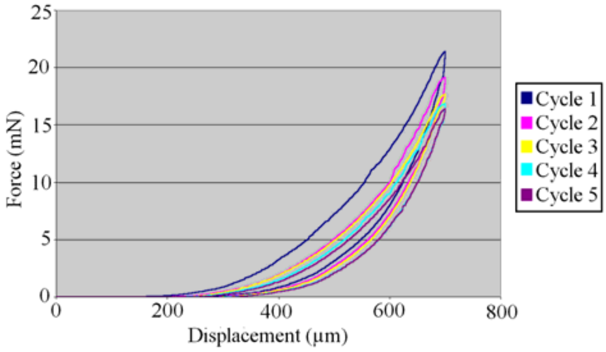

Figure 6: Loading-unloading curves for a cornea during five indentation cycles

A typical stress relaxation curve for an indented cornea is shown in figure 7. It can be seen that there was a substantial decrease in the force required to maintain the strain over time. This is typical in viscoelastic materials and suggests that the cornea is capable of adapting its internal structure to minimise the effect of the indentation.

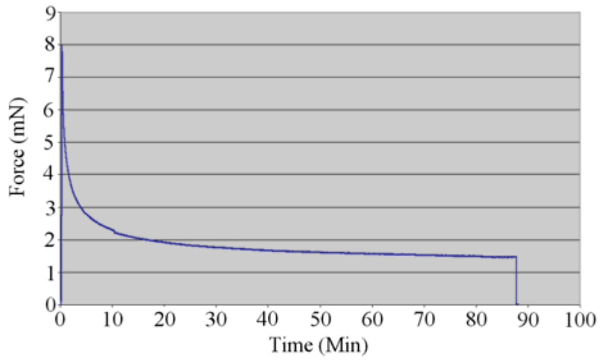

Figure 7: Force relaxation curve for a cornea subjected to a constant indentation displacement

Discussion

We have developed a new technique to examine the mechanical characteristics of human corneas. Previous techniques have either only examined uniaxial strain or have been complicated to use. Our indentation system is free of gripping problems, allows continuous biaxial force-displacement measurements for both loading-unloading with microscale resolution. It also allows the quantitative determination of both elastic and viscoelastic (time-dependent) properties of the cornea.

There are a number of potential applications of this system. The effect of different drugs or crosslinking agents on the mechanical and viscoelastic characteristics of the cornea can be easily assessed. This system can also be used to characterise the properties of artificial corneas and tissue engineered corneas. This could be extremely useful as previous studies suggest that tissue engineered corneas have significantly poorer mechanical strength than real human corneas [13]. Other applications include examining the effects of long-term storage on the corneas properties and examining the viability of animal corneas to model the characteristics of human corneas. The system also has the potential to be modified to examine the mechanical and viscoelastic properties of other soft human tissues such as skin or vascular tissue.

This method of mechanical characterization can be further developed to give a more comprehensive examination of the mechanical and viscoelastic characteristics of human cornea. A set of equations could be developed to describe the cornea during indentation as a non-linear non-isotropic material. Other properties such as the corneal curvature and the variation of corneal thickness at difference points should also be taken into account. Despite this the model described in this paper provides a simple and effective method for characterising the mechanical characteristic of corneas from indentation data.

Conclusions

We have demonstrated that indentation can be used to characterize the mechanical and viscoelastic properties of human cornea. This system should provide a more effective alternative to other methods of cornea characterization.

Acknowledgement

The OCT facility is supported by the University Hospital of North Staffordshire Cancer Research Fund.

References

[1] ANDREASSEN T. T., SIMONSEN A. H. AND OXLUND H. (1980): 'Biomechanical properties of keratoconus and normal corneas', *Exp. Eye Res.*, 31, pp. 435-441

[2] HOELTZEL D. A., ALTMAN P., BUZARD K. AND CHOE K. (1992): 'Strip extensiometry for comparison of the mechanical response of bovine, rabbit, and human corneas', *J. Biomech. Eng.*, 114, pp. 202-215

[3] ZENG Y., YANG J., HUANG K., LEE Z. AND LEE X. (2001): 'A comparison of biomechanical properties between human and porcine cornea', *J. Biomech*., 34, pp. 533-537.

[4] MAURICE D.M. (1988): 'Mechanics of the Cornea', in Cavanagh H. D. (Ed): 'The Cornea: Transactions of the World Cornea Congress III', (Raven Press Ltd., New York) pp. 187-193

[5] ELSHEIKH A. AND ANDERSON K. (2005): 'Comparative study of corneal strip extensometry and inflation tests', *J. R. Soc. Interface*, 2 117-185

[6] SHIN T. J., VITO R. P., JOHNSON L. W. AND MCCAREY B. E. (1997): 'The distribution of strain in the human cornea', *J. Biomech.*, 30, pp. 497-503

[7] JU B. F., LIU K. K., LING S. F. AND NG W. H. (2002): 'A novel technique for characterizing elastic properties of thin biological membrane', *Mech. Mat*., 34, pp. 749-754.

[8] WIRBELAUER C., SCHOLZ C., HOERAUF H., ENGELHARDT R., BIRNGRUBER R. AND LAQUA H. (2000): 'Corneal optical coherence tomography before and immediately after excimer laser photorefractive keratectomy', *Am. J. Ophthalmol*., 130, pp. 693-699.

[9] MUSCAT S., MCKAY N., PARKS S., KEMP E. AND KEATING D. (2002): 'Repeatability and reproducibility of corneal thickness measurements by optical coherence tomography', *Invest. Ophthalmol. Vis. Sci.,* 43, pp. 1791-1795

[10] AHEARNE M., YANG Y., EL HAJ A., THEN K. Y. AND LIU K. K. (2005): 'Characterizing the viscoelastic properties of thin hydrogel-based constructs for tissue engineering applications', *J. R. Soc. Interface*, in press

[11] STORM C., PASTORE J. J., MACKINTOSH F. C., LUBENSKY T. C. AND JANMEY P. A. (2005): 'Nonlinear elasticity in biological gels', *Nature*, 435, pp. 191-194

[12] FUNG Y. C. (1993): 'Biomechanics: Mechanical properties of living tissues, 2nd edition', (Springer_Verlag, New York)

[13] ORWIN E. J., BORENE M. L. AND HUBEL A. (2003): 'Biomechanical and optical characteristics of a corneal stromal equivalent', *J. Biomech. Eng.,* 125, 439-444

ISSN: 1727-1983 © 2005 IFMBE