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Measurement of Corneal Tangent Modulus using Ultrasound Indentation

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Abstract

Purpose: To develop a novel ultrasound indentation method to quantitatively assess the corneal biomechanical properties.

Methods: An ultrasound indentation probe consisting of a load cell as force sensor and a miniature ultrasound transducer as indenter was used to detect force-indentation relationship of cornea. The key idea was to utilize the ultrasound transducer to compress the cornea at a given indentation rate and to measure the corneal deformation with the eyeball overall displacement compensated. Thirteen corneal phantoms with Young's modulus ranging from 0.12 to 1.4 MPa were fabricated for the validation of measurement with reference to an extension test. In addition, fifteen fresh porcine eyes were measured by the system at the IOP of 15 and 30 mmHg. During the measurements, the probe was driven by a motorized stage. The tangent moduli of the phantoms and porcine cornea were calculated based on the obtained force-indentation data. Repeatability, coefficient of variation (CV), and intra-class correlation coefficients (ICC) of three repetitive measurements were determined to demonstrate the feasibility of this new measurement method.

Results: The tangent moduli of the corneal phantom calculated using the ultrasound indentation data agreed well with the results from the tensile test of corresponding phantom strips ($\mathrm{y} = 0.88\mathrm{x} + 0.15, R^2 = 0.94$). The mean tangent moduli of porcine corneas measured by the current system were 88 ± 27 kPa at 15 mmHg and 220 ± 53 kPa at 30 mmHg, respectively. The repeatability, CV and ICC of tangent modulus obtained by ultrasound indentation were 35 kPa, 14% and 0.77 at 15 mmHg; and 52 kPa, 8.6% and 0.87 at 30 mmHg, respectively.

Conclusions: The preliminary study showed that it is feasible to use ultrasound indentation to measure the corneal tangent modulus with good repeatability and the obtained moduli of silicone cornea phantoms well agreed with tensile test results. The results demonstrated that the ultrasound indentation can be potentially used for the corneal biomechanical properties measurement.

Keywords: Corneal biomechanics; Corneal tangent modulus; Ultrasound indentation

Introduction

Cornea is a transparent tissue which provides about 70% of the eye's optical refraction . The geometric topology of cornea includes shape, thickness and multilayer microstructure that determine refractive effects of the eye. Recently, refractive surgery by Laser-Assisted in Situ Keratomileusis (LASIK) is a popular treatment for correction of refractive errors by changing the thickness of cornea. However, the iatrogenic keratoconus after LASIK surgery has received much attention as the most serious complication of the surgery all along with its popularity in the world[1]. Due to the cutting of stroma, keratoconusis may potentially be induced immediately or sometime after the surgery among the preoperative keratoconus suspects.

Corneal biomechanical properties were reported to have a close relationship with the pathologies of some corneal degeneration diseases like keratectasia[2], keratoconus and pellucid marginal degeneration, as well as common eye disorders such as glaucoma, myopia and presbyopia. The collagen composition and structure of corneal stroma are the controlling factors of corneal biomechanical properties[3, 4]. Due to the high tensile stiffness of collagen fibrils and spatially varying distribution of lamellae in the stroma, cornea is a tissue with highly nonlinear, anisotropic, heterogeneous, and viscoelastic characteristics, both laterally and in depth[5]. Evaluations on corneal biomechanical properties include the measurements of corneal rigidity, viscosity, and elasticity. Many efforts have been devoted to the development of measuring techniques on corneal biomechanical properties, for example, conventional stress-strain measurement[6] and inflation test[7]. However, these methods are not easy to apply to the biomechanical measurement of cornea *in vivo*. Ocular Response Analyzer (ORA, Reichert, Depew, New York) and Corneal Visualization Scheimpflug imaging (Corvis ST, Oculus, Wetzlar, Germany) based on corneal dynamic response to applied air puff, have been introduced for clinical tests during the last decade[8, 9]. While ORA has been used for many research studies, it cannot provide an intrinsic mechanical parameter for cornea. The parameter "hysteresis" provided by ORA represents the air puff pressure difference between the inward and outward applanations, which is determined not only by the mechanical properties of cornea, but also cornea thickness, geometry, eyeball size, etc. On the other hand, Scheimpflug imaging technique is severely limited by geometrical and optical distortions which make careful corrections necessary beyond the quantitative extraction of biomechanics related information[10]. Recently it has been theoretically verified that a comprehensive correction would be required to obtain an accurate deformation measurement of the cornea using this technique[11].

Corneal biomechanical properties are also closely correlated with the intraocular pressure (IOP) measurement, which is the essential indicator for the diagnosis and management strategy of glaucoma, one of the leading causes of irreversible blindness. The traditional measurement methods on IOP are based on the classical Imbert-Fick principle, such as Goldman applanation tonometry (GAT)[12], TonoPen[13], dynamic contour tonometry

(DCT)[14]. But none of them can provide the corneal elasticity measurement. It has been recently reported that corneal mechanical indentation combining a load sensor and a linear actuator could be used to measure corneal tangent modulus on porcine and rabbit corneas[15]. Using the mechanical indentation, the displacement of cornea surface (calculated from the movement of the head of the indenter driven by the linear actuator) was treated as the corneal deformation, however, the result may not be accurate as any movement of the whole eyeball under the applied force would be counted into the corneal deformation. Therefore, additional development is required to solve this inherent issue of the mechanical indentation method.

Ultrasound indentation is a potential technique which can solve the bulk movement issue for accurate deformation measurement during the corneal indentation test. In ultrasound indentation the probe normally consists of a load cell connected in series with an ultrasound transducer as the indenter[16]. The tissue thickness and indentation deformation can be measured by ultrasound signal reflected from internal tissue interface. This method has been widely used for the stiffness measurement of soft tissue *in vivo* such as residual limb[17], diabetic foot[18], post-radiotherapy fibrotic neck[19] and lower back[20]. However, few studies have been reported in the literature on the use of ultrasound indentation for corneal biomechanical measurement. Normally, the size of ultrasound transducer is too big to be applied on cornea with curvature. This problem can be solved by adopting a small profile ultrasound transducer. In addition, as the ultrasound indentation uses the internal tissue interface as the reference for the extraction of deformation during indentation, the measurement result will not be affected by the bulk movement of the tested tissue such as the eyeball in corneal testing, which improves the measurement accuracy. Therefore, ultrasound indention has the potential for measuring corneal biomechanical properties with improved accuracy compared to traditional indentation. In this paper, we developed an ultrasound indentation method using a portable ultrasound indentation system with a miniature ultrasound transducer to measure corneal tangent modulus. For method validation, a series of tissue mimicking phantoms with different stiffness were fabricated and tested with ultrasound indentation, and the results were compared to those by a reference extension test. Preliminary tests were also conducted on 15 porcine corneal eyeballs in vitro in order to demonstrate the feasibility of the proposed testing method on corneal biomechanical test.

Materials and methods

Ultrasound indentation system

The developed ultrasound indentation probe was similar to that in our previous reports[16-18] except that a small profile ultrasound transducer was adopted for proper operation on cornea. An ultrasound transducer with a frequency of 10 MHz and a diameter of 3 mm was used to build a hand-held pen-sized indentation probe. A compressive load cell was connected in series to the ultrasound transducer as a force sensor to record the indentation force. The information of cornea such as the anterior chamber depth and corneal displacement was extracted by the ultrasound signals during indentation. A custom-designed program developed by Microsoft VC++ was responsible for data collection and analysis in which the force and ultrasound signal were used to calculate corneal biomechanical properties. All the data were recorded during experiment and further analyzed offline to obtain the biomechanical parameters.

Phantoms and porcine eyes preparation

Silicone phantoms were fabricated to mimic the structure of cornea. The phantoms were designed with a central thickness of 500 μm and a peripheral thickness of 1000 μm, respectively. The curvature radius of the anterior surface was 8 mm and that of the aspherical posterior surface changed from 6 mm to 7 mm consequently in the direction from center to periphery. The diameter of corneal part was designed to be 12 mm and the depth of the unloaded anterior segment was 3.8 mm (Figure 1). To mimic different corneal elasticities under different physiological and pathological status or among different subjects, corneal phantoms with different elasticity were fabricated by adjusting the silicone-oil ratio from 1:0.1 to 1:3.5. Corresponding silicone strips were also made with the same silicone-oil ratios as the phantoms. These silicone strips with different elasticity would be tested under standard testing machine and the measured mechanical parameters would be compared with those by the ultrasound indentation test to validate the developed measurement method. For system validation, a total of 12 silicone phantoms were successfully fabricated to be tested by the ultrasound indentation system.

Fifteen fresh porcine eyeballs were acquired from a local slaughterhouse and maintained in 0.7% normal saline at 4°C before testing. To reduce the swelling of cornea, all the specimens were tested in no more than 6 hours after death.

Experiments on corneal phantoms and porcine eyeballs

The setup for the phantom test is shown in Figure 2. The corneal phantom was mounted on an artificial anterior chamber which was connected to a motor driven pumping device for automatic intraocular pressure (IOP) control as indicated by the height change of saline solution. The change of IOP was detected by a pressure sensor (MPX5100, Freescale semiconductor Inc., Arizona, USA). For observing the effect of IOP, different IOP levels from 10 to 30 mmHg with increment of 5 mmHg were applied to the cornea at a fixed indentation rate of 50 mm/min. The maximum indentation was controlled as 1 mm. Prior to the test, trial tests with an indentation rate between 10 mm/min and 200 mm/min showed that they had negligible effects on the final results, so only a fixed indentation speed of 50 mm/min was adopted for the phantom test. In addition, a strip extensiometry test (Figure 3) was conducted to obtain the tensile modulus (tensile E) of the corresponding strips for comparison with the results of ultrasound indentation test. The strips were pre-stressed by placing samples with initial length of 30 mm under slight tension and then tested to a strain of approximately 3% elongation. Tensile E was estimated based on the equation[23]

$$E = \frac{T/A}{\zeta\ /L} \tag{1}$$

where T is axial load and ζ is the elongation of strip, A and L are the cross-sectional area and original length of the strip, respectively.

For the porcine eyeball test, the experimental setup is shown in Figure 4. The porcine eyeball was taken out and mounted on an eye-size cup which was used to simulate an eye socket to hold the eyeball. A needle was inserted into the anterior chamber of eyeball and connected to a saline-filled tube with the IOP detector and controller. The corneal central thickness and curvature at the IOP of 15 mmHg were measured by using corneal tomography (Pentacam, Oculus, Wetzlar, Germany). The porcine cornea was moisturized by a humidifier at room temperature during the indentation test. Typical ultrasound signals from the phantom and real porcine eyeball test are shown in Figure 5, where the interface reflected from the bottom of the artificial chamber in phantom test and the lens in porcine eyeball test, respectively, is clearly shown. Before porcine eye experiment, the eyeball was loaded and unloaded between 10 and 40 mmHg of IOP for 3 cycles to achieve pre-conditioning. Two IOP levels of 15 mmHg and 30 mmHg simulating normal and glaucomatous eye conditions were set to measure the corneal tangent modulus. The ultrasound indenter was adjusted to align with the corneal apex and moved forward to be in contact with the cornea using a small pre-load. After that, the motor stage drove the indenter to load the cornea at an indentation rate of 50 mm/min with a given depth of 1 mm. Five circles of loading-unloading were carried out for a test, and the last four cycles of load-displacement curves in the test were selected for data analysis. For each eyeball, three repetitive tests were performed and the average of the extracted parameters was used to represent the tissue properties. Standard deviation (SD) and coefficient of variation (CV) were used to represent the test repeatability under the ultrasound indentation test. For a better understanding of the bulk motion effect, the corneal biomechanical properties calculated from deformation based on the ultrasound signal and those based on the motor stage movement would be compared.

Calculation of corneal tangent modulus

As the indenter size was 3 mm in diameter, when the displacement was larger than 0.15 mm (based on eyeball geometry), the area between indenter and cornea was considered as full contact. According to the previous method, the corneal tangent modulus (tangent E) could be derived from the differential of force balance equation and determined by[15]

$$E|_{IOP} = \frac{a(R_c - t/2)\sqrt{1-v^2}}{t^2}\frac{dF}{d\delta}|_{IOP} \quad (2)$$

where, R_c and t are the radius of corneal curvature and the corneal central thickness, respectively. F is the indentation force, δ is the indentation depth, ν is the Poisson's ratio which was set to be 0.45 assuming the cornea is a nearly incompressible tissue under the selected indentation rate. The differential of force and indentation depth at a specific IOP was obtained from the force-displacement curve with the interval of $0.2 < \delta < 0.4\ mm$. And a is a geometry constant which can be determined from μ together with Table 1[24]

$$\mu = r_0 \left[\frac{12(1-\nu^2)}{(R_c - t/2)^2 t^2}\right]^{1/4} \quad (3)$$

where, r_0 is the radius of the contact area between the ultrasound indenter and the cornea. Generally, R_c and t change less than 5% in the tested IOP range so the measured values at a single IOP can be used to calculate tangent modulus E in Equation (2) at different IOP levels. After μ was calculated, the value of a could be obtained by interpolating the values in Table 1 with a three order polynomial spline interpolation.

Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, Illinois, USA) and MedCalc 13.0 (MedCalc Software, Ostend, Belgium) were used to conduct statistical analysis. Kolmogorov-Smirnov test was applied to estimate the normality of distribution of the measured variables. Welch's modified student's two-sample *t*-test and the Wilcoxon rank-sum test were used to determine differences in biomechanical parameters among groups. Linear regression analysis was performed to assess the relationship between tensile E and tangent E obtained at IOP of 10 mmHg and an indentation rate of 50 mm/min. P value less than 0.05 ($P < 0.05$) was considered as statistically significant. Repeatability analysis was carried out on the tangent E calculated from ultrasound and motor stage at the IOP of 15 mmHg and 30 mmHg, respectively. The analysis was conducted to calculate three repetitive measurements of fifteen porcine eyeballs accomplished by a single operator. Repeatability, coefficient of variation, and intra-class correlation coefficients (ICC) were calculated as the parameters of repeatability analysis[25]. ICC was interpreted as follows: when < 0.75: poor to moderate repeatability; 0.75-0.90: good measurement repeatability; and when > 0.90: excellent repeatability for clinical measures[26].

Results

The estimated tensile E values of the corneal phantoms ranged from 0.09 to 1.4 MPa. As shown in Figure 6, there is a significantly high correlation between the phantom tensile E measured by extension test and the corneal tangent E determined by the ultrasound indentation test ($y = 0.96x, R^2 = 0.96, P < 0.001$). Figure 7 shows the relationship between the measured tangent modulus and IOP in four typical corneal phantoms. The measured corneal tangent modulus was affected by the IOP changing from 10 to 30 mmHg. For the corneal phantoms with different tensile E, The trend of effect was the same. When the IOP increased, the measured corneal tangent modulus increased.

For the porcine eye test, the average central corneal thickness and radius of curvature were 1.19 ± 0.05 mm and 8.34 ± 0.17 mm, respectively. Corneal force-displacement curves at two IOPs of 15 mmHg and 30 mmHg were obtained by the ultrasound indentation with an indentation rate of 50 mm/min. An exponential fitting of the force-deformation relationship was applied first and the fitting in the deformation ranging from 0.2 to 0.4 mm was used to extract the slope of force-deformation for calculation of tangent modulus using Equation (2) (Figure 8). The repeatability, CV and ICC of tangent modulus calculated by ultrasound indentation were 0.035 MPa, 14.353 and 0.765 (at 15 mmHg); 0.052 MPa, 8.580 and 0.870 (at 30 mmHg); for motor stage, the corresponding values were 0.027 MPa, 13.102 and 0.787 (at 15 mmHg); and 0.043 MPa, 8.851 and 0.896 (at 30 mmHg), respectively (Table 2). Figure 9 shows the comparison between two types of deformations measured using different methods in order to show the advantage of using ultrasound indentation for deformation measurement. The first method treated the motor stage displacement directly as the deformation. The second method used the deformation measured from the ultrasound signals under the IOP of 15 mmHg and 30 mmHg, respectively. The displacements measured by motor stage and ultrasound were different which increased with the increase of motor travel range. Furthermore, this difference changed with the change of IOP. For the porcine eye experiment, the mean corneal displacements calculated by ultrasound indentation were 0.85 ± 0.07 mm at IOP of 15 mmHg and 0.79 ± 0.06mm at IOP of 30 mmHg compared with the indentation depth of 1 mm for the motor stage. The values of tangent moduli calculated from motor stage were 0.070 ± 0.02 MPa at 15 mmHg and 0.17 ± 0.05 MPa at 30 mmHg, while the mean porcine corneal tangent moduli measured by ultrasound were 0.089 ± 0.026 MPa at 15 mmHg and 0.220 ± 0.053 MPa at 30 mmHg, with an increase of 27% and 29% respectively.

Discussion

A novel ultrasound indentation system has been developed for the measurement of corneal biomechanical properties. For system validation, the tangent moduli of corneal phantoms measured by ultrasound indentation at IOP of 10 mmHg were consistent with the results from tensile moduli of corresponding strips determined by stress-strain test. For the corneal phantoms with different elasticity, when the IOP increased, the tangent modulus would increase correspondingly. Thus, this preliminary study on corneal phantoms showed that the current ultrasound indentation system had the ability to distinguish corneas with different tangent moduli. For the test on porcine eye *in vitro*, two IOPs of 15 and 30 mmHg were set to simulate the eye with normal and glaucoma. The mean corneal tangent moduli calculated from the current system were 0.089 ± 0.026 MPa at 15 mmHg and 0.220 ± 0.053 MPa at 30 mmHg, which were consistent with some previous studies[27, 28].

In our test, a flat-end ultrasound transducer with a frequency of 10 MHz and a diameter of 3 mm was used as the indenter. The ultrasound speed in eye was considered as 1560 m/s and the theoretical axial resolution was approximately 120 µm[29], which is enough to separate echoes from the two interfaces of porcine cornea. If this technique is used for animal with thinner cornea, ultrasound transducers with a higher frequency can be used. The ultrasound echo from the lens surface was tracked for cornea deformation measurement. The radio-frequency ultrasound signal was sampled at 100 MHz, which represented a time-resolution of 10 ns. Accordingly, the resolution for cornea deformation was approximately 0.016 mm. In order to detect corneal displacement more accurately, higher sampling rate for collecting ultrasound signals can be used. According to the structure of cornea, the full contact between cornea and indenter tip would occur when the cornea was compressed into about 0.15 mm by using the indenter with a diameter of 3 mm. To ensure a full contact, a part of the force-displacement curve was selected for the calculation of corneal tangent modulus when the displacement was between 0.2 and 0.4 mm. The optimal diameter of the ultrasound transducer needs to be further investigated, as an indenter with too small size may punch the cornea to cause damage, but one with too large size may require a larger deformation for a full contact with cornea, leading to potential damage as well.

It was noted that the corneal tangent moduli obtained by deformation from motor stage were smaller than the values determined by deformation from ultrasound signals (decrease by 21% and 23% at 15 and 30 mmHg, respectively). Because ultrasound could collect the echo from the lens surface, the actual displacement of cornea was calculated by the relative distance from cornea to lens in the ultrasound indentation. Generally, the displacements calculated from ultrasound were smaller than the values directly from motor stage movement. The main reason was the overall movement of eyeball during the indentation. According to ocular anatomy, the eyeball is contained and protected by the eye socket with associated muscles, vessels, and nerves[30]. The muscles of the eye are responsible for the stability and movement of the eye. In other words, the eyeball is not rigidly fixed into the eye socket and is supported by other tissues. If the eye is indented by an external force, it is possible that the whole eyeball would have some backward movement together with corneal displacement. Similar finding was reported by other researchers. Corneal contour would have deformation when the cornea was applied by an air puff generated by Corvis ST device[31]. In the current experiment, we found that the eyeball moved backward slightly when the ultrasound indenter deformed the cornea. The mean movement of eyeball was approximately 0.15 mm at 15 mmHg and 0.21 mm at 30 mmHg when the total displacement from the motor stage was 1 mm. In other words, there was 21% (under 30 mmHg IPO) of deformation of cornea was wrongly counted if the displacement of cornea surface measured by the movement of motor was used as the cornea deformation. Due to the effect of IOP, the eyeball became stronger to withstand external force with the increase of IOP. So the movement of eyeball at 15 mmHg was smaller than that at 30 mmHg. Therefore, the deformation cornea should be calculated based on the cornea movement related to a the displacement of a reference target which moves along the whole eyeball movement. It was demonstrated in this study that the displacement of the lens surface measured from the ultrasound signal fulfilled this requirement if the relative position of lens in the eyeball was assumed to be fixed during the indentation on the cornea. Therefore, it was assumed that the mechanical properties measured from the ultrasound indentation were more accurate compared to conventional corneal indentation where the corneal deformation was obtained by indenter displacement[15]. Because of the anatomical position of lens and a small indentation depth, the assumption of lens stability was reasonable during the indentation. Considering the anatomy of eye, we believe that the results obtained from the porcine eyes tested in vitro would be applicable to tests in vivo. Nevertheless, the stability of the lens with respect to the eyeball in vivo during indentation warrants further investigations in future study.

In summary, a novel ultrasound indentation method has been proposed to measure the corneal biomechanics. The results on corneal phantoms and porcine eyes have demonstrated that this system is able to quantitatively assess the tangent modulus of cornea with improved measurement accuracy, because of the compensation for the eyeball movement. We expect that the ultrasound indentation approach could potentially become an assistive tool for the determination of corneal biomechanics in ophthalmologic investigation of pathologies such as glaucoma and keratoconus, which are still difficult to diagnose in early stage. Further studies would be necessary to further demonstrate the technique in vivo.

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Figure Captions:

Fig.1. Design of silicone corneal phantoms and a sample of silicone strip with the same stiffness as the phantom

Fig.2. The schematic of the ultrasound indentation system for corneal biomechanical properties measurement

Fig.3. The strip extensiometry test of the corneal phantom

Fig.4. The experimental setup of the porcine eye test for corneal tangent modulus measurement using the ultrasound indentation

Fig.5. Typical ultrasound signals obtained from (a) corneal phantom and (b) porcine eyeball for the extraction of initial thickness and deformation

Fig.6. Correlation between the tensile modulus measured by the extension test and the tangent modulus determined by the ultrasound indentation with an IOP of 10 mmHg at an indentation rate of 50 mm/min.

Fig.7. Change of measured tangent modulus with IOP in four corneal phantoms with tensile E ranging from 0.09 to 1.40 MPa

Fig.8. Typical curves of indentation force and deformation obtained under two different IOPs (15 and 30 mmHg) in a porcine eyeball test. The slope of the fitted curves in the

deformation range of 0.2 to 0.4 mm is used to calculate the tangent modulus

Fig.9. Comparison of deformation curves determined with different methods under two different IOPs, i.e., motor stage displacement and ultrasound signals at IOP of 15 mmHg and 30 mmHg

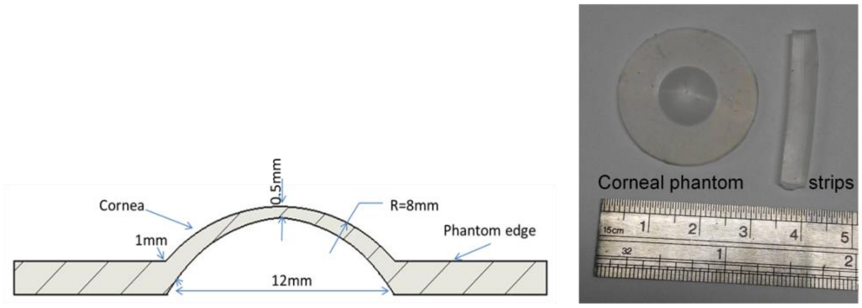
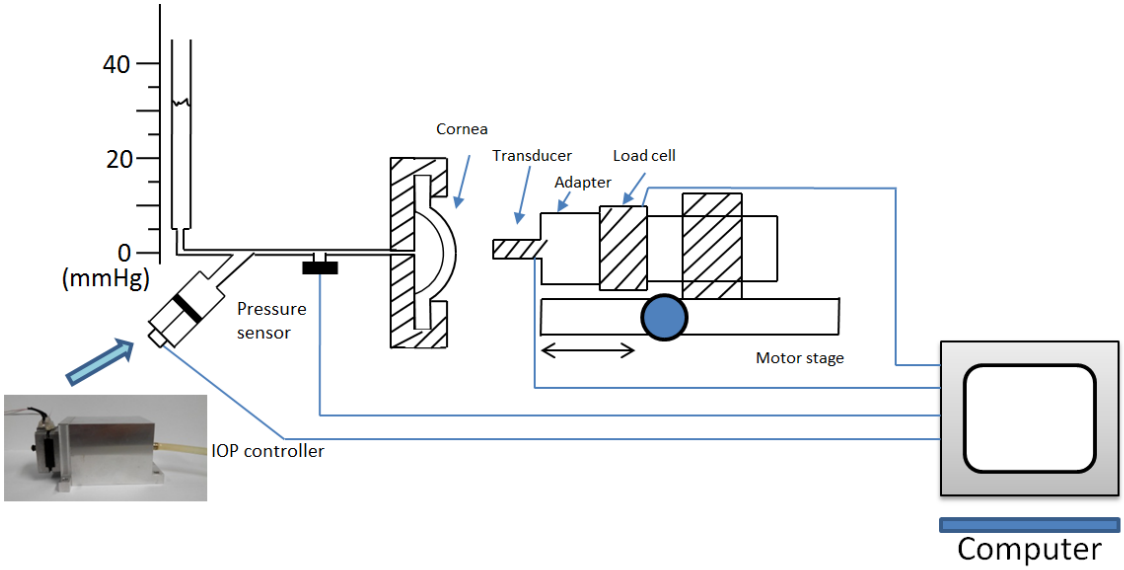

Cornea
0.5mm
R=8mm
1mm
Phantom edge
12mm
Corneal phantom
strips

Figure 1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20
0
(mmHg)
Cornea
Transducer
Load cell
Adapter
Pressure
sensor
Motor stage
IOP controller
Computer

Figure 2

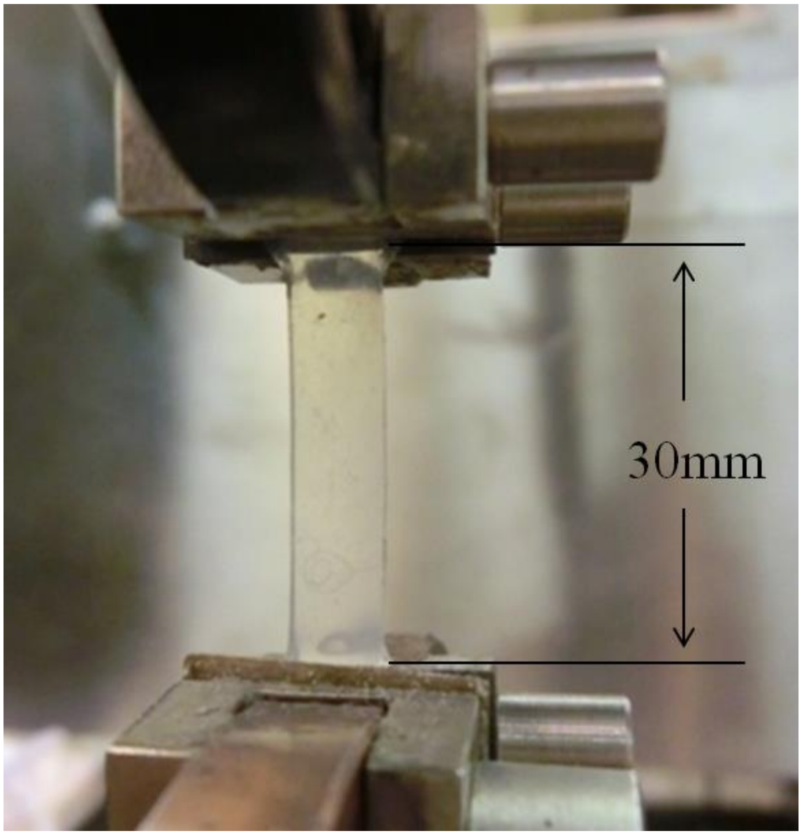


Figure 3

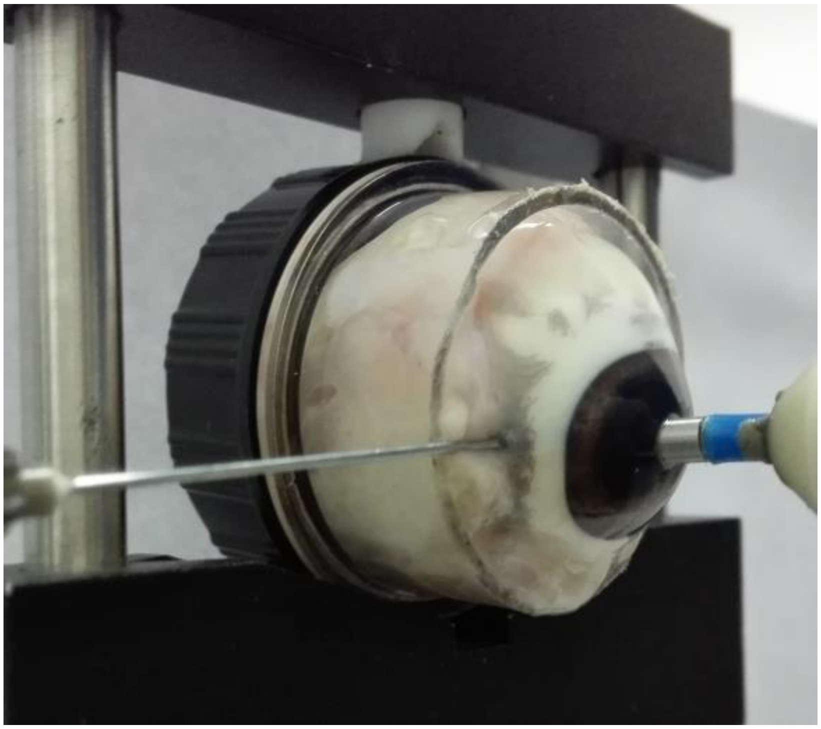
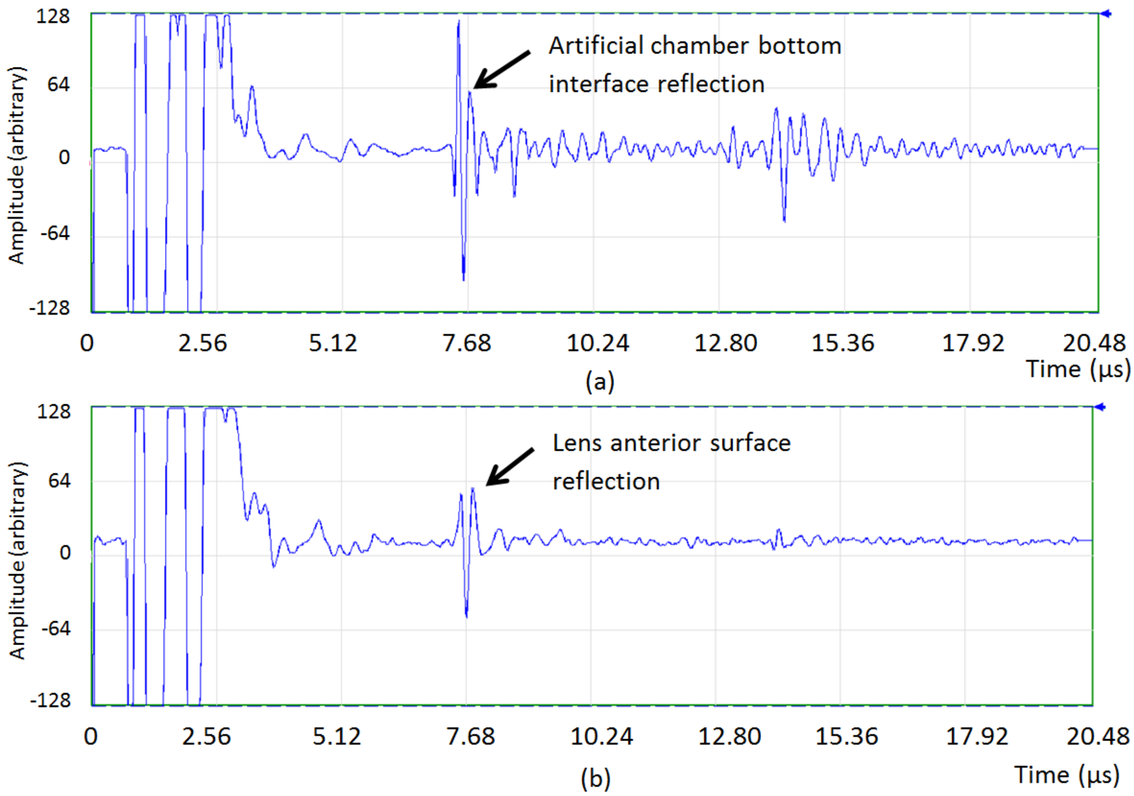


Figure 4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64
0
-64
-128
Amplitude (arbitrary)
0
2.56
5.12
7.68
10.24
12.80
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17.92
20.48
Time (µs)
Artificial chamber bottom interface reflection
(a)
128
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-64
-128
Amplitude (arbitrary)
0
2.56
5.12
7.68
10.24
12.80
15.36
17.92
20.48
Time (µs)
Lens anterior surface reflection
(b)

Figure 5

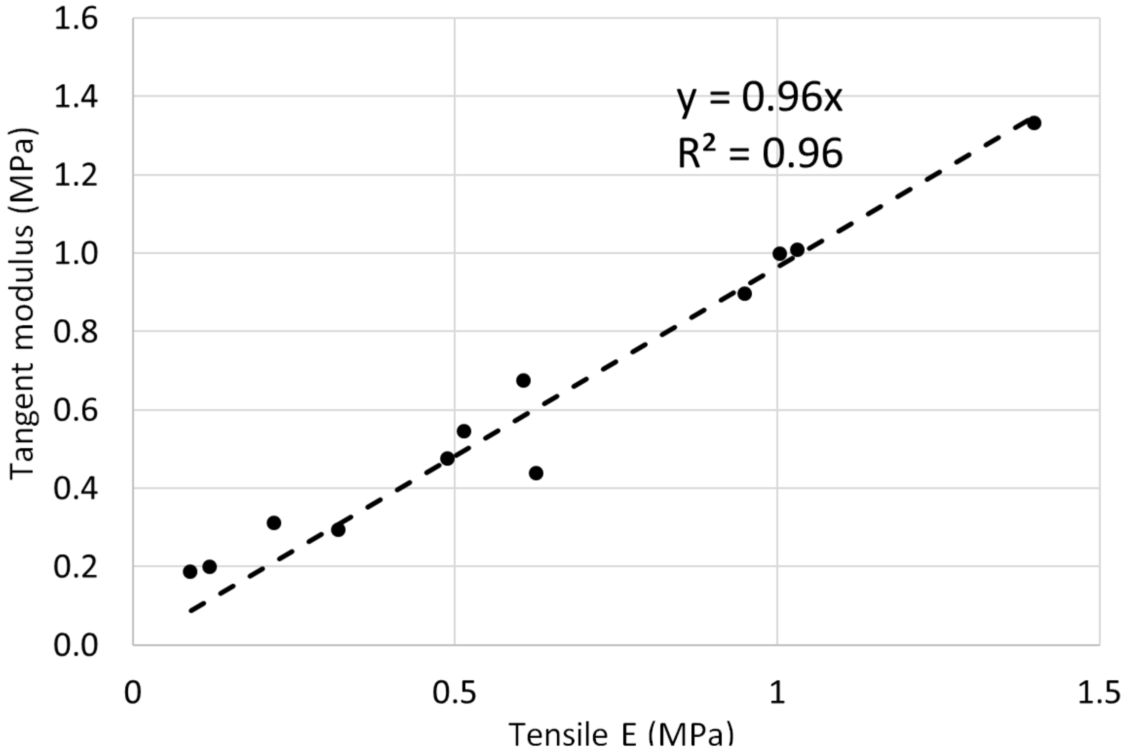
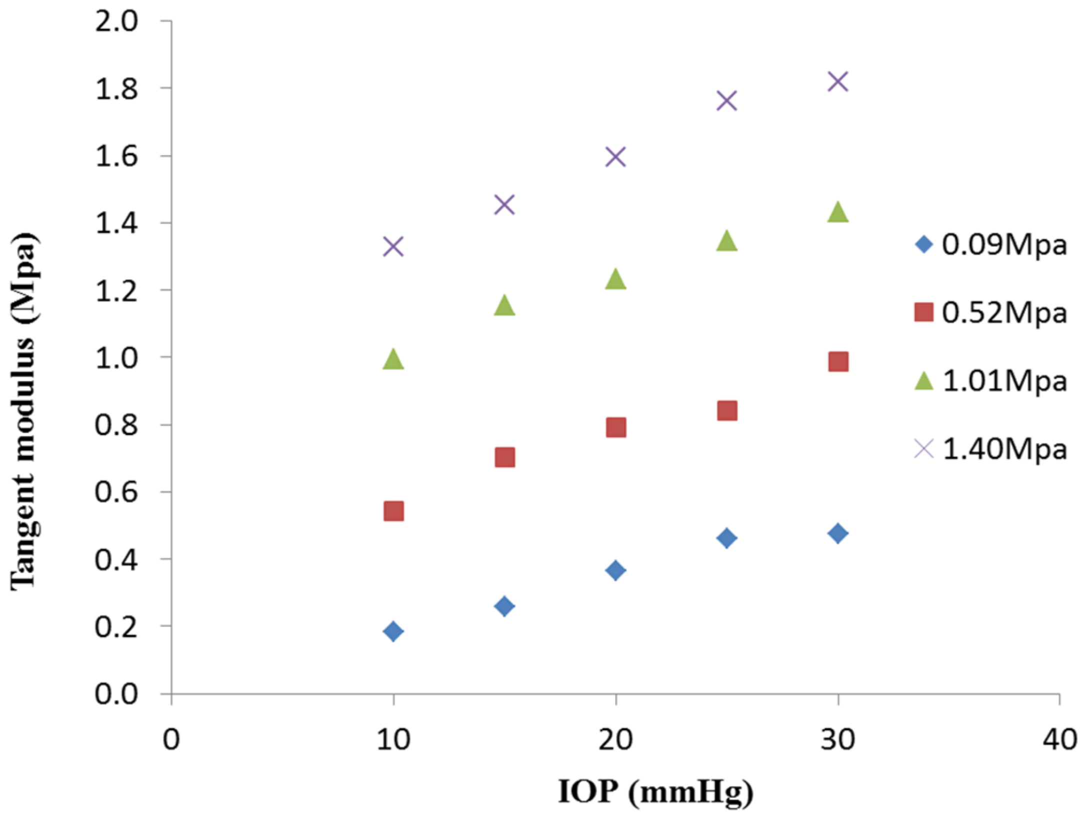
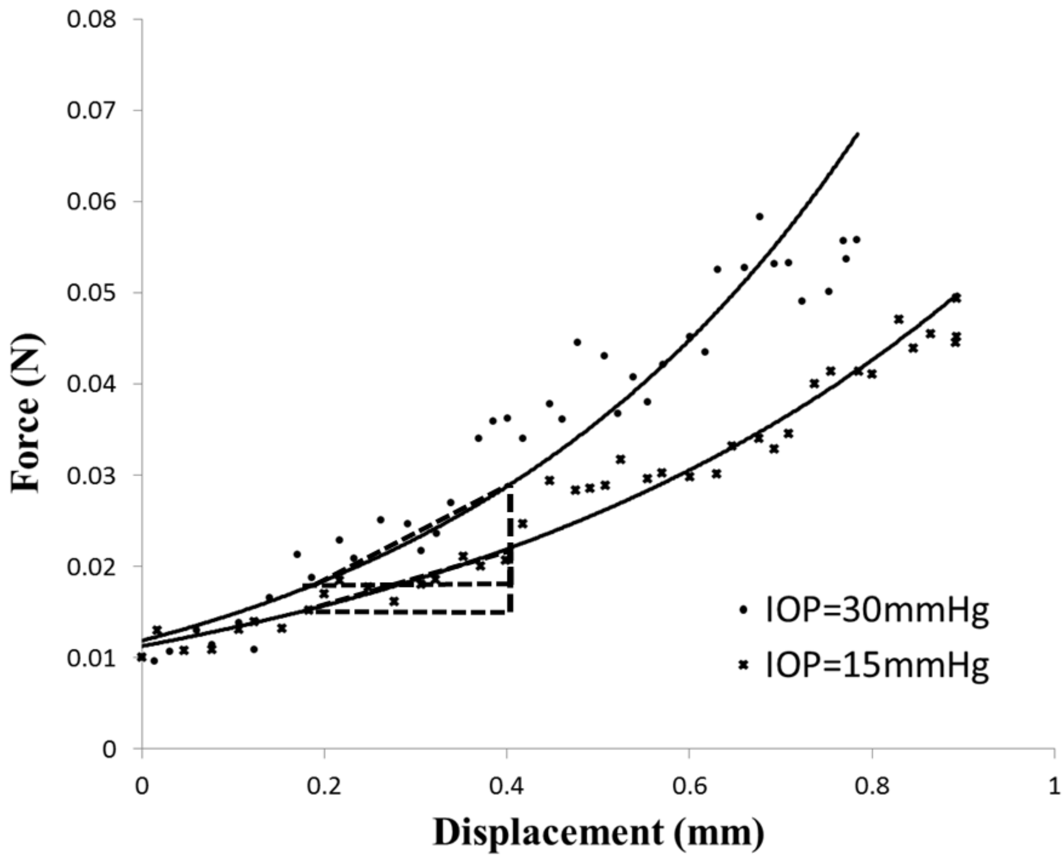

1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Tangent modulus (MPa)
y = 0.96x
R² = 0.96
0
0.5
1
1.5
Tensile E (MPa)

Figure 6



2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
Tangent modulus (Mpa)
IOP (mmHg)
0.09Mpa
0.52Mpa
1.01Mpa
1.40Mpa

Figure 7



0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Force (N)
0
0.2
0.4
0.6
0.8
1
Displacement (mm)
IOP=30mmHg
IOP=15mmHg

Figure 8

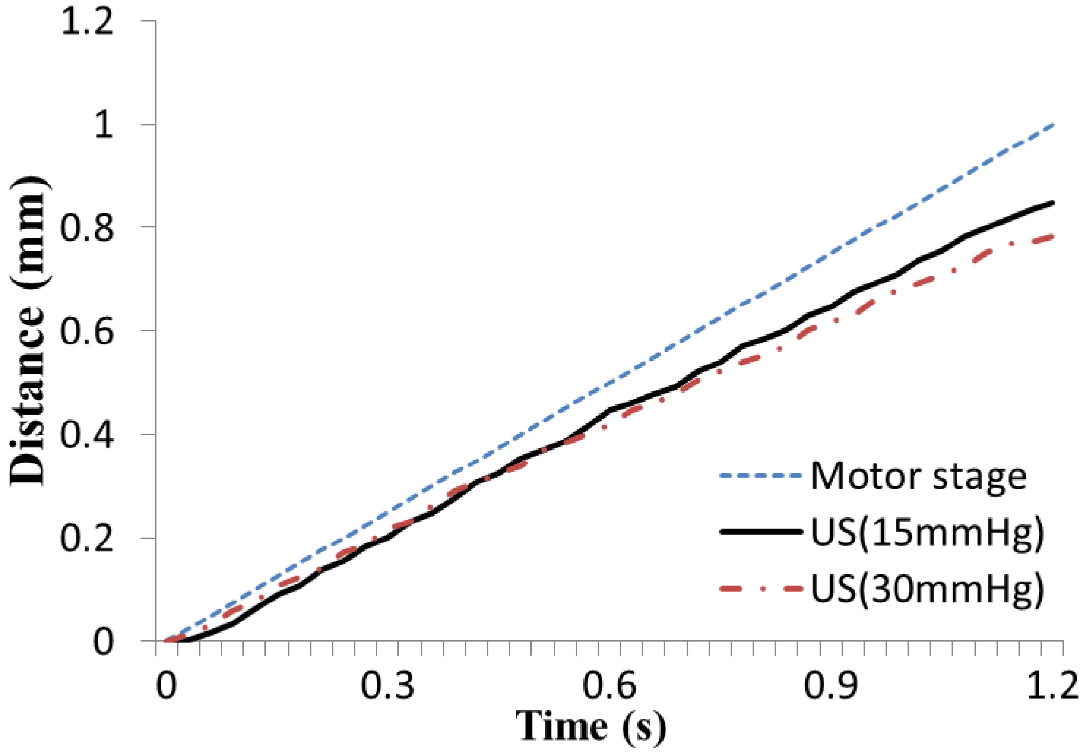

Distance (mm)
1.2
1
0.8
0.6
0.4
0.2
0
0
0.3
0.6
0.9
1.2
Time (s)
Motor stage
US(15mmHg)
US(30mmHg)

Figure 9

Table Captions:

Table1. Relationship between factor *a* and parameter μ as used in Equation (2) and (3).

Table2. Repeatability of the E parameters for the three repeated measurements (n = 15).

Table 1

μ	0	0.1	0.2	0.4	0.6	0.8	1.0	1.2	1.4
a	0.433	0.431	0.425	0.408	0.386	0.362	0.337	0.311	0.286

Parameters	**Repeatability (MPa)**	**CV(%)**	**ICC**
Ultrasound E(MPa) 15 mmHg	0.035	14.353	0.765
Motor stage E(MPa) 15 mmHg	0.027	13.102	0.787
Ultrasound E(MPa) 30 mmHg	0.052	8.580	0.870
Motor stage E(MPa) 30 mmHg	0.043	8.851	0.896

CV: coefficient of variation; ICC: intraclass correlation coefficient