Optical coherence tomography measurements of the fresh porcine eye and response of the outer coats of the eye to volume increase

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Abstract. Corneal and scleral thickness and anterior chamber dimensions are required for understanding developmental and pathological processes. Parameters of the eyeball are also required to calculate optical and material properties. As the eyeball resembles a pressure vessel, it has been suggested that elasticity of the cornea and sclera could be calculated from the measurements of thickness. Baseline corneal and scleral thicknesses and anterior chamber dimensions and how these change with incremental increases of intraocular fluid are measured in fresh porcine eyes using the Visante OCT (optical coherence tomography). At baseline, corneal thickness is almost constant. Anterior scleral thickness is variable, decreasing from 0.91 ± 0.07 mm near the limbus to a minimum of 0.58 ± 0.13. Posterior scleral thickness is more constant with an average of 0.78 ± 0.09 mm. Near the optic nerve the thickness increases to 1.00 ± 0.09 mm. Average baseline anterior chamber angle, diameter, and depth were found to be 33.15 ± 4.91 deg, 13.60 ± 0.38 mm, and 2.13 ± 0.22 mm, respectively. After fluid injections, maximum changes in corneal and scleral thicknesses were 9 to 10 and 1 to 3%, respectively. Anterior chamber angle and depth decreased slightly but significantly. Changes in the eyeball coats with fluid injections, indicate that the pressure vessel model can be applied to the eye to calculate corneal and scleral elasticities. © *2008 Society of Photo-Optical Instrumentation Engineers.* [DOI: 10.1117/1.2907453]

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1 Introduction

The thickness of the outer coats of the eye is an important biometric measure for a number of research and clinical purposes: for studies on the growth of the eyeball, for investigating changes in disease states, and for ascertaining response to induced experimental conditions required in the measurement of material properties. In the clinic and in studies conducted *in vivo*, it is only possible to study the anterior sections of the eye. Measurement of scleral thickness has thus far required slicing the eyeball after freezing or fixation. Temperature changes and/or use of chemicals will have an effect on the tissue, and it is not certain how much error this introduces to the parameter being measured. Similarly, measurements of rheology of the cornea and/or sclera have been conducted using methods that may alter the properties being measured (slicing, freezing, and thawing), and this may explain the lack of consistency in findings.[1–3] Ideally, material properties of the cornea and sclera should be measured on fresh whole eyeballs. Such approaches have been used previously.[4,5] These studies have approximated the eyeball to a pressure vessel capable of radial expansion with progressive injections of fluid to measure elasticity and rigidity. The theory of pressure vessels requires that certain assumptions about the expansion of the vessel and the thickness of its walls are met.[6] The thickness must be less than 20 times the mean diameter of the eyeball (a condition that does apply to the eye), and the stress is assumed to be constant over the wall thickness.[6] Although the thickness of the outer coat of the eyeball is not constant, if the variations are small enough not to cause differences in stress and hence disproportionate changes in thickness with expansion, the pressure vessel model can be applied to the eye to estimate elasticity of the tissues that constitute the outer coat of the eyeball. The thickness of the cornea and sclera can be measured using optical coherence tomography (OCT).

OCT was developed for imaging the retina using interferometry to compare the delay and intensity of light backscattered from a sample to a reference beam that has traversed a known path length with specified time delay. The first tomographic image of a sample was obtained by Huang et al.,[7] who used a fiber optic interferometer with a reference mirror that

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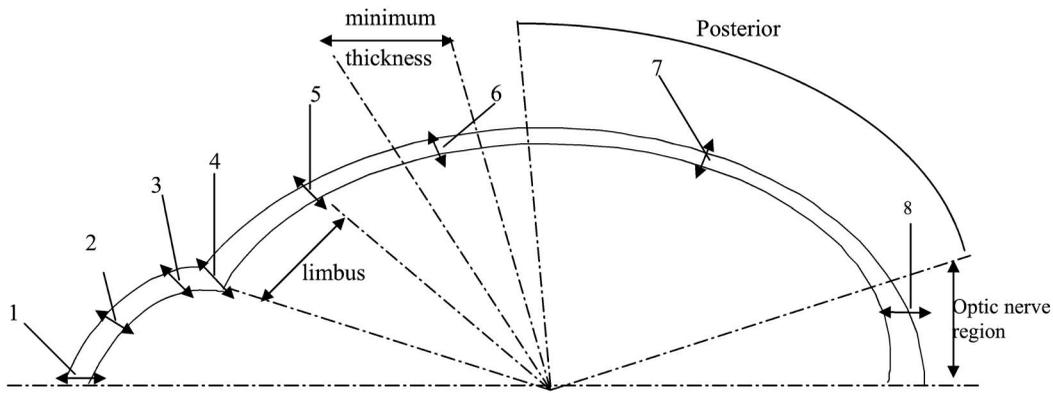
Fig. 1 Schematic cross section of globe of the eye indicating location of measurements.

moved at the requisite high speed to perform low-coherence interferometric scans. The signals from these scans were used to produce the tomographic image. This technique was further developed to incorporate a dual-beam system and to image, for the first time, the *in vivo* optic disc.[8] The technique continued to improve and systems for imaging not just features of the retina and its supporting structures, but also those of the anterior eye were developed[9] and are now commonly used in the clinical setting.[10] The Visante OCT uses light at 1310 nm to produce high-resolution images of the anterior segment and to calculate the biometric parameters of the cornea and anterior chamber.[11]

The method of OCT for imaging various features of the eye is not restricted to just the clinic; a prototype instrument was shown to be effective in imaging the anterior chamber angle and parts of the ciliary body in enucleated porcine eyes.[12] This study uses the Visante OCT to measure the thickness of the cornea and sclera in fresh whole porcine eyeballs and to determine changes in thickness of the outer coats of the eye and the dimensional changes of the anterior chamber in response to incremental injections of saline.

2 Methods

Twenty-four porcine eyes, used in this study, were obtained from the local abattoir; collection was within 4 h of death. All eyes were from animals aged between 5 and 6 months. Twelve eyeballs were used to measure thickness of the cornea, the diameter, depth, and angle of the anterior chamber; the other twelve were used to measure the thickness of the sclera. Experiments were completed between 3 and 6 h postmortem; each experiment lasted no longer than 10 min. During the experimental procedure, samples were kept moist with saline solution to prevent dehydration. Extraocular musculature and extraneous fat were removed from each eyeball before OCT imaging.

Eyeballs were placed on a specially designed holder[5] fixed onto the chin rest of the Visante OCT system (Carl Zeiss Meditec, Inc). The instrument has an optical axial resolution of 18 μm (in the tissue), a lateral resolution of 60 μm, and a 16-$\times$6-mm scan field. The holder ensured that eyeballs were maintained in a secure position during imaging and measurement,[5] allowing only rotation of the eyeball in an axis perpendicular to the longitudinal plane. Positions at which

measurements were made are shown in Fig. 1. This diagram shows positions of measurements made on the combined sets of samples: the twelve eyeballs on which corneal and anterior chamber measurements were made and the twelve eyeballs used for measuring the thickness of the sclera (from the corneal limbus to the optic nerve).

A syringe clamped to the side of the tube was used for injecting fluid into the optic nerve without disturbing eyeball position,[5] and this also ensured that measurements were made around the same plane (the longitudinal plane). The changes in corneal thickness and anterior chamber angle, depth, and diameter in response to intraocular fluid increases were measured on the twelve eyeballs used to determine baseline corneal thickness and anterior chamber dimensions. Similarly, the changes in scleral thickness, in response to fluid increase, were measured on the twelve eyeballs for which baseline scleral thickness had been determined. Images were acquired

Table 1 Baseline OCT measurements, mean±SD (standard deviation).

	Mean	±SD
1[a]	0.96	0.05
2[b]	0.95	0.04
3[c]	0.97	0.05
4[d]	1.00	0.04
5[e]	0.91	0.07
6[f]	0.58	0.13
7[g]	0.78	0.09
8[h]	1.00	0.09

[a]central corneal thickness (mm);
[b]midcorneal thickness (mm);
[c]peripheral corneal thickness (mm);
[d]thickness of the limbus on corneal side (mm);
[e]thickness of limbus on scleral side (mm);
[f]minimal thickness region of anterior sclera (mm);
[g]posterior scleral thickness (mm);
[h]posterior scleral thickness (mm) within 5 mm of the edge of the optic nerve.
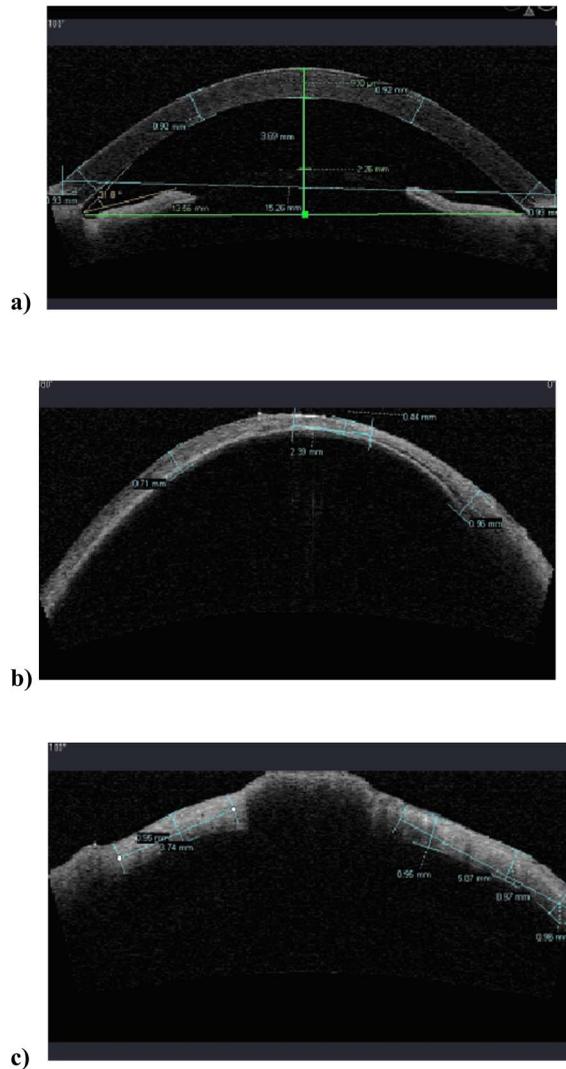
a)

b)

c)

Fig. 2 Tomographic images of (a) the anterior chamber, (b) anterior and posterior sclera, and (c) sclera near the optic nerve.

after each of five consecutive injections of 100 μl of saline. The biometric parameters were evaluated using the analysis mode of the Visante OCT system software. Experiments were performed at an ambient temperature of 20 °C. Eyeballs were weighed before and after experimentation. The final eyeball weights were within 8% of their baseline weights, as determined by subtracting the weight of the injected intraocular fluid from the weight of the eyeball at the end of the experiment.

3 Results

The baseline thicknesses of the cornea and sclera in fresh porcine eyes are shown in Table 1 (corresponding locations of measurement are shown in Fig. 1). The baseline thickness of the cornea is relatively constant: 0.96 ± 0.05 mm at the center (point 1, Fig. 1), 0.95 ± 0.04 mm at the midpoint (point 2, Fig. 1), and 0.97 ± 0.05 mm in the periphery (point 3, Fig. 1) [Table 1, Fig. 2(a)]. At the limbus, the corneal thickness increases slightly to an average of 1.00 ± 0.04 mm (Table 1) decreasing on the scleral side of the limbus to an average of
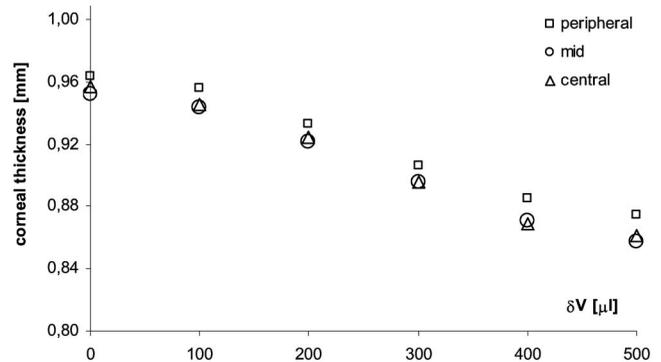

Fig. 3 Corneal thickness (in millimeters) plotted against the incremental change in volume (δV) (in microliters).

0.91 ± 0.07 mm (Table 1). The thickness of the anterior sclera gradually decreases over an arc length of 1.92 ± 0.20 mm to an average minimum thickness of 0.58 ± 0.13 mm [Figs. 1 and 2(b)]. This region of marked decrease in scleral thickness covers 7% of the total sclera. The posterior section of the sclera is more constant in thickness than the anterior section and has an average thickness of 0.78 ± 0.09 mm over 45% of the total sclera. At a distance of 4.51 ± 0.69 mm from the edge of the optic nerve, the posterior scleral thickness increases to a maximum of 1.00 ± 0.09 mm. This scleral thickness is maintained to the closest measurable point of $1.65 + 0.52$ mm from the optic nerve [Fig. 2(c)].

At baseline, the anterior chamber angle is 33.15 ± 4.91 deg and the average anterior chamber diameter is $13.60 + 0.38$ mm. The depth of the anterior chamber, measured as the distance from the posterior cornea to the anterior lens, is 2.13 ± 0.22 mm [Fig. 2(a)].

After incremental injections of saline, the thicknesses of the central, mid, and peripheral cornea decrease slightly (Fig. 3). Standard deviations for all points range from 0.04 to 0.06 (the error bars are not shown in Fig. 3 because the superimposition of three sets of error bars on the same points along the x axis makes it difficult to distinguish one set from another). The incremental decrease in corneal thickness (central, mid, and peripheral) is around 0.01 mm after the first and last incremental injections and is greatest (0.03 mm) after the third and fourth injections. The overall decrease in central and midcorneal thickness values, after the final injection of 500 μl is 10% of the initial thickness value. The overall peripheral thickness decreases to 9% of the initial peripheral thickness. These changes are statistically significant ($p < 0.001$).

The limbal and scleral thicknesses show smaller changes than the cornea after injections of saline. Thickness changes after injections were measured at all points except point 8 (near the optic nerve) because the presence of the injecting device made imaging of this point difficult. Any decreases in scleral thickness between baseline and after final injection of 500 μl of fluid, are less than the standard deviations and vary from 1 to 3% from baseline thickness (Table 2).

The anterior chamber angle decreases with incremental increase in fluid (Fig. 4). The difference between the average value of the angle at baseline and after maximum injection of

Table 2 The mean±SD of corneal and scleral thickness at baseline and after incremental intraocular injections of saline. The locations of points 4 to 7 are shown in Fig. 1.

Injection of fluid (μ)	Thickness						
	Central Corneal (mm) (point 1)	Mid Cornea (mm) (point 2)	Peripheral Cornea (mm) (point 3)	Limbus—Corneal Side (mm) (point 4)	Limbus—Scleral Side (mm) (point 5)	Sclera—Minimal Thickness Region (mm) (point 6)	Posterior Sclera (mm) (point 7)
Baseline	0.96±0.05	0.95±0.04	0.97±0.05	1.00±0.04	0.91±0.07	0.58±0.13	0.78±0.09
100	0.95±0.05	0.94±0.04	0.96±0.05	0.99±0.04	0.91±0.08	0.57±0.13	0.78±0.09
200	0.92±0.05	0.92±0.05	0.93±0.06	0.99±0.04	0.90±0.08	0.57±0.13	0.77±0.08
300	0.90±0.05	0.90±0.05	0.91±0.05	0.98±0.04	0.90±0.07	0.57±0.14	0.77±0.09
400	0.87±0.06	0.87±0.05	0.89±0.06	0.99±0.08	0.89±0.07	0.56±0.14	0.77±0.08
500	0.86±0.05	0.86±0.05	0.87±0.06	0.97±0.05	0.89±0.07	0.57±0.14	0.77±0.08

500 μl (33.15 ± 4.91 and 28.23 ± 4.20 deg, respectively) is statistically significant ($p < 0.002$). The change in diameter of the anterior chamber (Fig. 5) from its baseline of 13.60 ± 0.38 to 14.07 ± 0.48 mm, following the maximum fluid injection, was not statistically significant ($p > 0.05$). There was a statistically significant decrease ($p < 0.001$) in anterior chamber depth from baseline of 2.13 ± 0.22 to 1.99 ± 0.25 mm after the maximum injection of 500 μl.

4 Discussion

The thickness of the outer coats of the porcine eye, as indeed those for eyes of other species, has thus far been measured using histological methods such as freezing or fixation. These methods alter the material properties of tissues and this may have an effect on tissue size. The OCT system provides highly resolved measurements of anterior segment structures, including thickness of the cornea and anterior sclera as well as depth, angle, and diameter of the anterior chamber of the *in vivo* human eye. This study has further exploited the imaging capacity of this instrument by measuring the thickness of the entire outer coat of the eye as well as features of the anterior chamber in fresh porcine eyes *in vitro*.

The results show that the variations in thicknesses of the porcine cornea and sclera are less than those observed in human[13–15] and monkey eyes.[16] Unlike human and monkey corneae, the thickness of the porcine cornea does not show significant variations. The average OCT thickness at the center of the porcine corneae (0.96 ± 0.05 mm) measured in this study is comparable to *in vitro* ultrasound biomicroscopic (UBM) measurements of corneae from fresh porcine eyes (0.98 mm).[17]

The posterior sclera, which comprises almost half of the total sclera, has a fairly constant average thickness to within 5 mm from the optic nerve, where scleral thickness increases to approximately 1 mm. Anterior scleral thickness varies significantly more than the rest of the sclera; however, the variations are smaller than those measured following formalin fixation.[18] Fixation has been shown[19] to increase the varia-
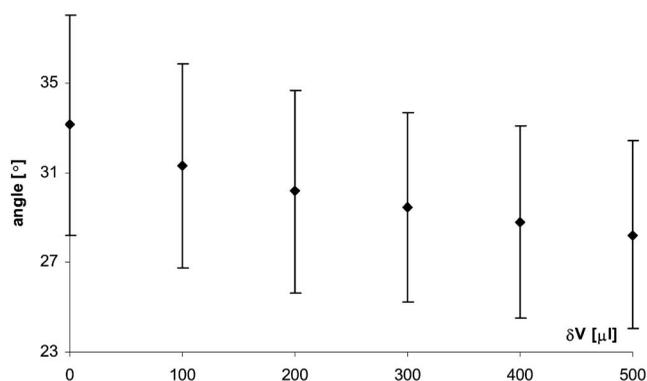
Fig. 4 Anterior chamber angle (in degrees) plotted against the incremental change in volume (δV) (in microliters).
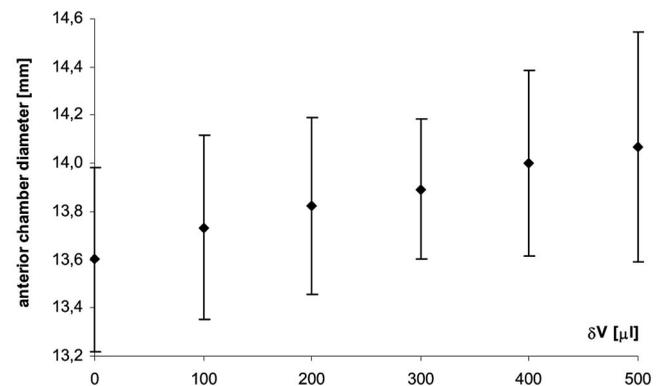
Fig. 5 Anterior chamber diameter (in millimeters) plotted against the incremental change in volume (δV) (in microliters).

tions in thickness in different parts of the sclera in canine and equine eyes by over 50%.

The baseline porcine anterior chamber depth was recorded as 2.13 ± 0.22 mm. Note that the OCT uses the optical path length and converts this to its geometrical counterpart, dividing the optical path length by the refractive index value of 1.376. As the anterior chamber is filled with aqueous, which has a refractive index of 1.333, the more accurate value for the anterior chamber depth is 2.20 ± 0.23 mm. This is very close to the value of 2.28+0.18 mm obtained from *in vitro* measurements of fresh porcine eyes using Scheimpflug photography[20] but slightly smaller than the measurement of 2.47 mm on fresh porcine eyes found[17] using UBM. The latter study also reported an anterior chamber angle of 30.45 deg, which is within the range of values found in this study (33.15 ± 4.91 deg).

Incremental intraocular injections of 100 μl did not cause significant changes in the thicknesses of either cornea or sclera. The maximum volume of 500 μl of fluid resulted in a significant decrease of 9 to 10% in the thickness of the cornea and smaller, nonsignificant decreases in scleral thickness (between 1 and 3% of the initial thickness). Following intraocular fluid injections, neither the cornea nor the sclera showed any significantly varying or disproportionate changes in thickness in any sections along their respective lengths. In view of these findings, and the fact that the baseline cornea and posterior sclera have relatively constant thickness values, the pressure vessel model can be applied to the eyeball to estimate corneal and scleral elasticity.[6] The cornea and posterior sclera should be treated as separate tissues in the calculations.

With incremental injection of fluid, the anterior chamber angle and depth decreased slightly, while the diameter of the anterior chamber increased, indicating that with increase in fluid volume in the eyeball, the cornea is stretched in the plane of the limbus. An increase in intraocular fluid will result in a rise in intraocular pressure (IOP). From a previous study, in which the change in IOP with fluid increase was measured in porcine eyes,[5] the injection of 100 μl increments of fluid caused the IOP to rise by about 6 mm Hg (from a baseline of 16 mm Hg): the final value after five incremental injections reached just under 50 mm Hg. The pressure/volume relationship of the globe was found to be approximately linear over the range of fluid volume injected intraocularly.[5]

A more recent study considered the effect of increasing pressure on exised human corneae.[21] Samples were mounted in a sealed artificial anterior chamber and saline injected at a steady rate. Using time-domain OCT, the measured decrease in corneal thickness was found to be between 112 and 120 μm in response to a pressure increase of 200 mm Hg, while the radius of curvature increased by 247 ± 106 μm. Approximately the same decrease in central corneal thickness (100 μm) was observed in this study, in response to a volume increase of 500 ml (which was found to correspond to an IOP value of around 50 mm Hg). The comparable decrease in corneal thickness between the two studies demonstrates that IOP has only a minimal effect on corneal thickness. These observations[21,22] add further support to the concept that the cornea acts to buffer and protect the eye from sharp increases in IOP.

5 Conclusion

The anterior chamber OCT can be exploited to measure the biometry of the whole eyeball in fresh samples, alleviating the need for fixation, freezing, and cutting. The eyeball can tolerate an increase of 500 μl of fluid without causing disproportionate changes in thickness along the lengths of both cornea and sclera. This finding together with the relatively constant thickness of the cornea and posterior sclera render the eyeball an appropriate system for application of the pressure vessel model for estimating corneal and scleral elasticity.

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