Quantifying changes in lenticular stiffness with optical coherence elastography

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ABSTRACT

Maintaining a normal intraocular pressure (IOP) is important for visual health. Elevated IOPs have been implicated in many diseases, such as glaucoma and uveitis. The effects of an elevated IOP on the delicate tissues of the optic nerve head and retina are well-studied, but there is a lack of information about the effects of high IOPs on the stiffness of the crystalline lens. Changes in lenticular biomechanical properties have been implicated in diseases such as presbyopia and cataract, therefore, measuring lenticular biomechanical properties is crucial to understanding the etiology and progression of the leading causes of vision impairment. Additionally, there has been even less research focused on the effects of storage media on lenticular stiffness. Previous studies have been focused on the "gold standard" of mechanical testing on excised lenses. However, mechanical testing is invasive and destructive, and removal of the lens from the eye-globe does not allow for properly replicating the lens environment in the eye-globe. Thus, there is a need for noninvasive measurement techniques capable of performing *in situ* and *in vivo* elastographic measurements of the lens. Here, we artificially controlled the IOP of whole porcine eye-globes (N=3). Acoustic radiation force induced low amplitude displacements (<10 μm) at the apex of the lenses, which then propagated as an elastic wave. The elastic wave propagation was detected by a phase-sensitive optical coherence elastography (PhS-OCE) system. The results show that the stiffness of the lenses increased when IOP increased from 10 mmHg IOP to 40 mmHg. Additional OCE measurements were made on excised lenses stored in various media (PBS, DMEM, and M-199) at different pHs (4-7) and at different temperatures (4°C, 22°C, and 37°C). The results show that the stiffness of the lenses increased slightly when incubated at 4°C or 22°C, but decreased when the lenses were incubated at 37°C, while lenses incubated in M-199 showed more stability in their stiffness than lenses incubated in PBS and DMEM. Moreover, the lenses stored in M-199 at a pH of 7 showed a decrease in stiffness over 24 hours, while the more acidic M-199 media caused an increase in lenticular stiffness.

Keywords: lens, stiffness, elasticity, optical coherence tomography, optical coherence elastography

1. INTRODUCTION

Normal intraocular pressure (IOP) is crucial for proper eye-globe geometry and health of delicate ocular tissues, and subsequently, visual acuity [1, 2]. Normal IOP in the human eye ranges from about 10 to 20 mmHg [3]. Many ocular diseases are correlated with an elevated IOP, which is also known as ocular hypertension (OHT), such as glaucoma and uveitis [4]. Changes in IOP manifest differently on different ocular tissues. For instance, the cornea and sclera can deform with the greater IOP, which can lead to myopia and keratoconus [5]. The cornea and sclera are easily accessible, but the crystalline lens is inside the eye-globe. Thus, it is far more difficult to measure the biomechanical properties of the lens in its natural state within the eye-globe. Therefore, investigations on the mechanical properties of the lens have been focused on extracted lenses and have primarily utilized the "gold standard" of mechanical testing. While these studies have elucidated a lot of useful information about lenticular biomechanical properties, such as how the lens

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stiffness increases with age [6, 7], these studies cannot replicate the environment and biomechanical conditions of the lens in its natural state. In addition, the storage medium, incubation temperature, and medium pH may affect the biomechanical evaluation during *ex vivo* measurements, which has been demonstrated with other tissues such as the cornea [8].

As mentioned previously, the majority of lens biomechanical assessments have been performed with mechanical testing. However, there is a need for noninvasive measurement techniques in order to preserve the integrity of the lens and replicate the lens environment. Ultrasound elastography (USE) [9, 10] and magnetic resonance elastography (MRE) [11] are clinically-available techniques that have been used to evaluate the biomechanical properties of the lens. However, their relatively poor spatial resolution and contrast as well as a need for contact-based excitation may not be appropriate for lenticular biomechanical assessments, particularly *in vivo*. Here, we utilized a noninvasive elastography technique, optical coherence elastography (OCE), which has sub-nanometer levels of displacement sensitivity with phase-resolved detection [12-14] and has been used previously to measure the biomechanical properties of the lens *in situ* [7, 15]. OCE was used (1) to assess the biomechanical properties of fresh porcine lenses *in situ* (N=3) while the eye-globe IOP was cycled; and (2) to measure the changes in lenticular stiffness of excised porcine lenses (N=30) that were stored at various media (PBS, DMEM, and M-199), at various temperatures (4°C, 22°C, and 37°C), and various pHs. Our results demonstrated that the stiffness of the crystalline lens increased along with IOP and that lower incubation temperatures or higher pH of M-199 medium also increased the lens stiffness.

2. METHODS AND MATERIALS

Figure 1 shows a schematic of the phase sensitive OCE (PhS-OCE) system for measurements of the effects of IOP on lenticular stiffness. The OCE system consisted of a spectral domain optical coherence tomography (SD-OCT) and acoustic radiation force (ARF) delivery systems. The SD-OCT system was based on a Michelson-type interferometer and utilized a superluminescent light diode (SLD) with a central wavelength of 840 nm and bandwidth of 49 nm. The acquisition speed of line scan camera in the spectrometer was 25 kHz. Acoustic radiation force induced low amplitude displacements (<10 μm) at the apex of the lenses, which then induced transversely propagating elastic waves. The elastic wave propagation was detected by the OCE system with M-B-mode imaging [16]. Successive M-mode images ($n = 251$) were acquired over a ~6.3 mm line, where the center of the scan and ARF excitation were at the apex of the lens. The group velocity of the elastic wave was determined by the slope of a linear fit of the wave propagation distances and the corresponding propagation times [17]. Whole fresh porcine eye-globes (Sioux-Preme Packing Co. IA, USA, N=3) were cannulated with two needles for IOP control. One needle was connected via tubing to a saline-filled syringe placed in a micro-infusion pump. The other needle was connected via tubing to the pressure transducer to form the closed-loop IOP system [18]. A Matlab (MathWorks, Natick, MA) GUI program was developed to control the closed-loop IOP system. The IOP was changed by infusion or extraction of saline from the syringe. The OCE measurements were made at IOPs of 10 mmHg to 40 mmHg at 5 mmHg increment.

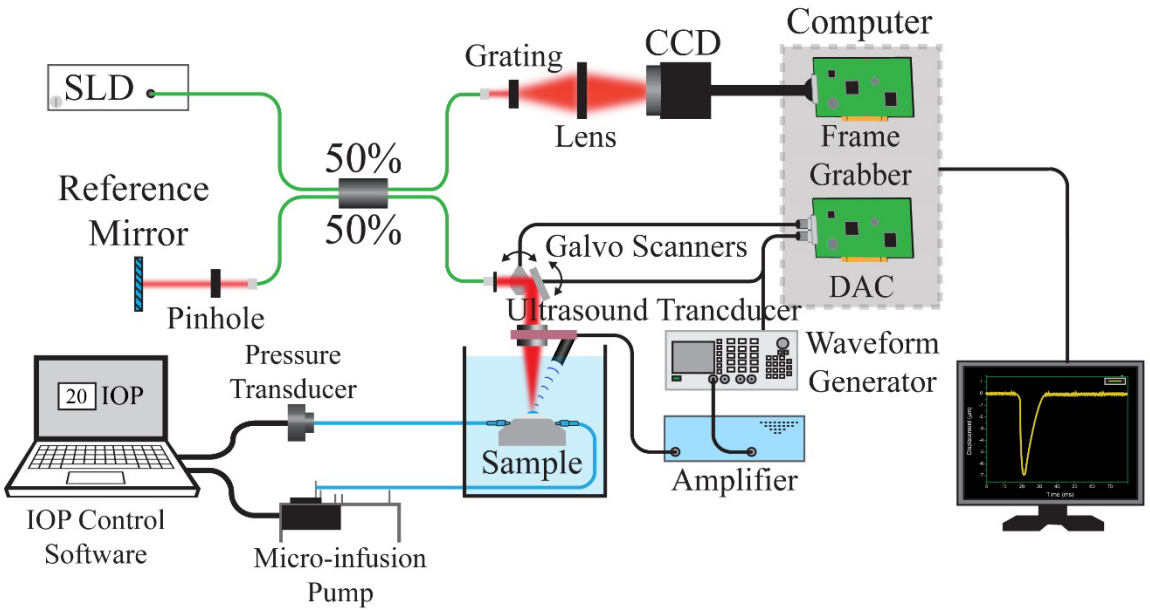

Figure 1. Schematic of the experimental setup during the *in situ* measurements of the effects of IOP on lenticular stiffness.

Figure 2 is a schematic of the experimental setup for measuring the effect of storage conditions on *ex vivo* porcine lenses in different storage media, temperatures and pH values. Here, a focused micro air-pulse produced the low amplitude displacement, which then induced the propagation of transverse elastic waves [19]. Whole lenses (n=30) were removed from fresh porcine eye-globes (Sioux-Preme Packing Co. IA, USA). The lenses were separated into three different groups. The first set of measurements was focused on assessing the effects of the storage medium (PBS, DMEM, and M-199) on lenticular stiffness. The lenses were placed into the corresponding medium (N=3 for each medium) and incubated for 24 hours at 37°C at a pH of 7.0. The effect of incubation temperature on lens stiffness was measured on the second group of lenses. The lenses were incubated in M-199 for 12 hours at 4°C, 22°C and 37°C at a pH of 7.0 (N=3 for each temperature). The effects of pH were investigated on the third group of lenses in M-199 medium. The lenses were incubated for 24 hours at 37°C in DMEM at a pH of 4.0, 5.0, 6.0, and 7.0 (N=3 for each pH). During each measurement, the pH value was measured by a pH meter (B10P, VWR International Co, PA, USA). One molar hydrochloric acid solution and NaOH solution were used to adjust the pH to the target value. Similar to the IOP measurements, M-B-mode imaging was performed to capture the elastic wave propagation where successive M-mode images ($n = 251$) were acquired over a ~6.1 mm line, where the center of the scan region and air-pulse excitation were at the apex of the lens. All OCE measurements were made before and after the appropriate incubation period.

In all studies, the group velocity of the elastic wave was determined by the slope of a linear fit of the wave propagation distances and the corresponding propagation times [17]. The Young's modulus, E, was estimated by the surface wave equation, $E = \frac{2\rho(1+\nu)^3}{(0.87+1.12\nu)^2} c_g^2$, where ρ=1.183g/L was the density [20], ν=0.5 was Poisson's ratio [21], and c_g was the OCE-measured elastic wave group velocity.

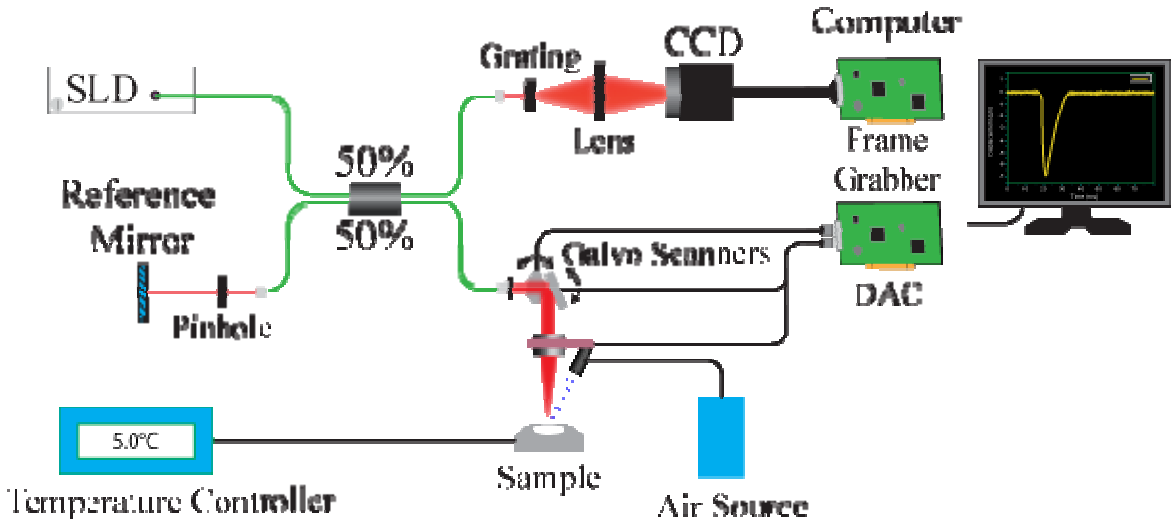

Figure 2. Schematic of the experimental setup while measuring the effect of storage medium on *ex vivo* lenticular stiffness.

3. RESULTS

Figure 3 plots the stiffness as estimated by the surface wave equation for each of the samples for different IOPs. The stiffness of the lenses grows as the IOP increases, and this trend was observed in all samples. The average Young's modulus of the lenses increased from 9.5 ± 0.9 kPa at 10 mmHg IOP to 12.2 ± 0.8 kPa at 40 mmHg IOP. This effect is likely the result of the deformation of the crystalline lens, and consequent exhibition of the nonlinear elastic properties of the lens. These results show the effect of stiffening with IOP for lens is significantly less noticeable than for cornea [9, 22]. The possible explanation of this difference is that the lens experiences less deformation during IOP elevation than cornea, such that nonlinear elastic properties of the lens do not play such a significant role as in the cornea.

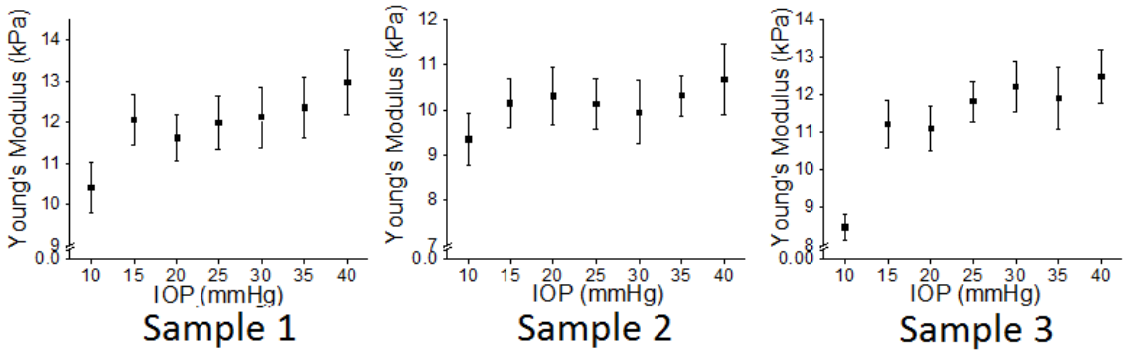

Figure 3. The estimated Young's moduli of three samples as functions of IOP.

Figure 4 shows the results of the estimated Young's modulus of the lenses in different media. The Young's modulus of the lenses that were incubated in PBS showed a dramatic increase over 18 hours, increasing from 7.3 ± 0.7 kPa to 13.0 ± 0.3 kPa. However, there was a slight decrease in lenticular stiffness after 24 hours of incubation as the comparison to 18 hours of incubation. After 12 hours, the lenses incubated in DMEM showed only a slight increase in stiffness, which is from 6.1 ± 0.6 kPa to 8.3 ± 0.2 kPa. However, there was a dramatic increase in stiffness to 13.7 ± 1.8 kPa and 11.6 ± 2.9 kPa after 18 and 24 hours of incubation, respectively. In the meanwhile, the lenses incubated in M-199 actually decreased in stiffness slightly over the 24-hour incubation period (from 8.3 ± 0.6 kPa at 0 hours to 7.0 ± 0.6 kPa at 24 hours). These results indicate that M-199 medium is a better storage medium than DMEM or PBS at preserving the porcine lens stiffness at 37°C over a period of 24 hours.

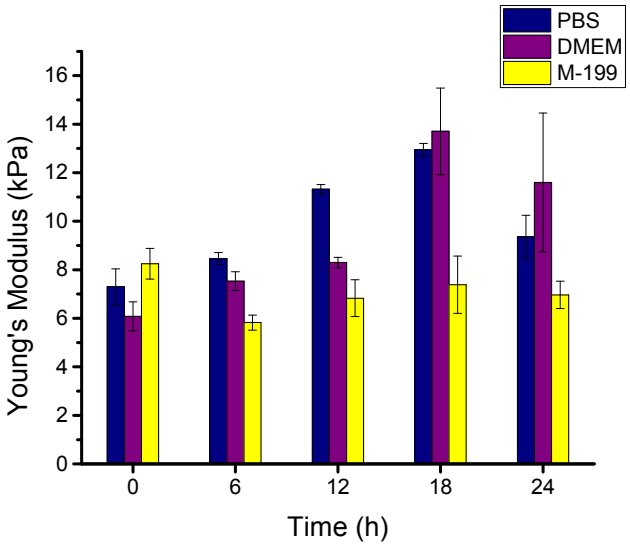

Figure 4. The estimated Young's modulus of the lenses incubated in the different media (N=3 for each medium) over 24 hours. The errors bars represent the inter-sample standard deviation.

While the stiffness of the lenses increased when incubated for 12 hours at 4°C and 22°C, when the lenses were incubated at 37°C the stiffness decreased. Figure 5 plots the Young's modulus of the lenses that were incubated at 4°C, 22°C, and 37°C. The stiffness of the lenses incubated at 4°C increased from 6.3 ± 0.4 kPa to 7.0 ± 0.2 kPa after 12 hours. The Young's modulus of the lenses incubated at 22°C for 12 hours increased from 6.0 ± 0.4 kPa to 6.8 ± 0.4 kPa. The Young's modulus of the lenses incubated at 37°C decreased from 5.5 ± 0.2 kPa to 4.4 ± 0.4 kPa after 12 hours.

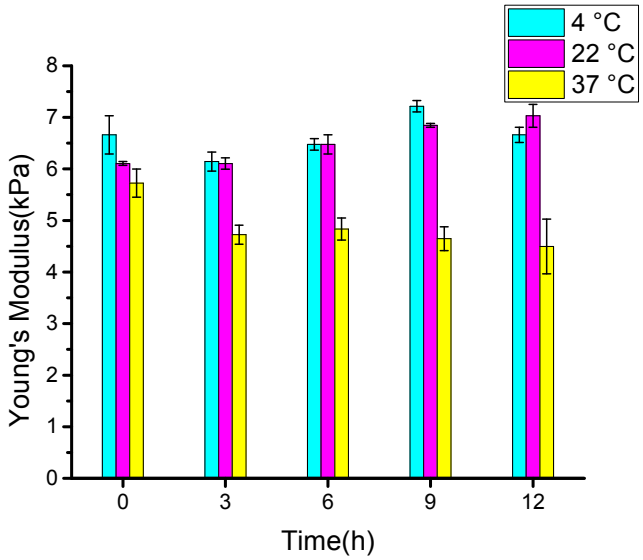

Figure 5. The estimated Young's modulus of the lenses incubated in the indicated media at 4°C, 22°C, and 37°C (N=3 for each temperature) over 12 hours. The error bars are the inter-sample standard deviation.

Figure 6 illustrates the change in Young's modulus of the porcine lenses in M-199 medium for different pHs (4.0, 5.0, 6.0 and 7.0) over 24 hours. The Young's modulus of the lenses incubated at pH=7.0 slightly decreased from 7.4 ± 0.8 kPa to 5.7 ± 1.5 kPa after 24 hours. However, the lenses stiffened when the pH was lowered, and the rate at which the lenses stiffened over time increased as the incubation medium pH decreased. The most dramatic increase in stiffness was

in group pH=4.0, where the stiffness changed from 7.1 ± 0.3 kPa to 9.3 ± 3.1 kPa from the pre-incubation measurement at 0 hours to 6 hours after incubation.

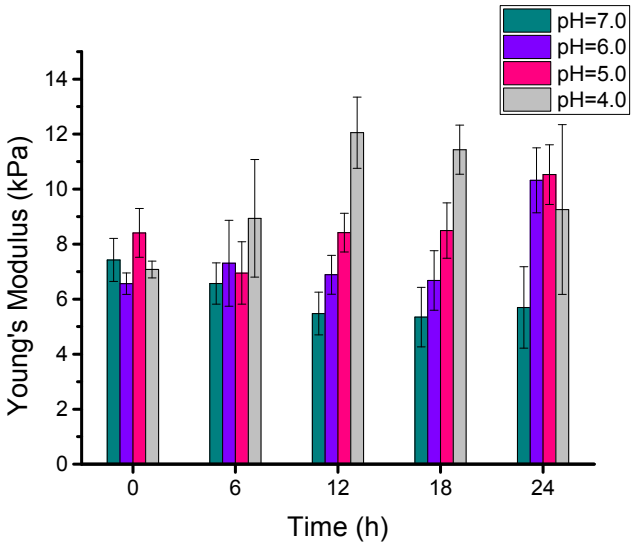

Figure 6. The estimated Young's modulus of the lenses incubated in M-199 at the indicated pH over 24 hours. (N=3 for each pH) over 24 hours. The error bars are the inter-sample standard deviation.

Further work is required to develop a more robust analytical wave models that can quantify the lens biomechanical properties accurately by incorporating the true lens geometry and boundary conditions, similar to our work on the cornea [23, 24]. Future work will also be focused on understanding the non-monotonic changes in lenticular stiffness as a function of IOP, medium, temperature, and pH level.

4. CONCLUSIONS

In this study, we evaluated the changes of *in situ* porcine lenticular biomechanical properties while artificially increasing IOP. The results showed an increase in lenticular stiffness as a function of IOP. We also evaluated the changes of *ex vivo* porcine lenticular biomechanical properties in different incubation media, incubation temperatures, and incubation medium pH levels. M-199, which is generally used to culture fibroblasts, preserved the stiffness of the lenses up to 24 hours better than PBS or DMEM. The lenses incubated at 37°C maintained their elasticity, but the lenses incubated at 4°C and 22°C showed a slight increase in stiffness, indicating some structural changes in the lens. Finally, the lenses incubated in M-199 at a pH of 7.0 showed almost no change in stiffness. However, the more acidic the storage medium was, the stiffer the lenses became after 12 to 24 hours, indicating that the acidic media caused structural damage to the lenses. Our results show that OCE could be used to noninvasively assess lenticular biomechanical properties and may be useful for studying lens biomechanical properties *in vivo*.

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