Assessing the effects of storage medium on the biomechanical properties of porcine lens with optical coherence elastography

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ABSTRACT

There has been a large amount of research focused on studying the biomechanical properties of the lens *ex vivo*. However, the storage medium of the lenses may affect the biomechanical evaluation during *ex vivo* measurements, which has been demonstrated with other tissues such as the cornea. In this work, we utilized a focused micro air-pulse and phase-sensitive optical coherence elastography to quantify the changes in lenticular biomechanical properties when incubated in different media, temperatures, and pHs for up to 24 hours. The results show that the lenses became stiffer when incubated at lower temperatures and higher pHs. Meanwhile, lenses incubated in M-199 were more mechanically stable than lenses incubated in PBS and DMEM.

Keywords: Optical coherence elastography (OCE), storage medium, stiffness, biomechanical properties, crystalline lens

1. INTRODUCTION

The changes in viscoelastic properties of the crystalline lens play an important role in the onset and progression of diseases and conditions such as cataract and presbyopia [1, 2]. There has been a large amount of research focused on the biomechanical properties of the lens [3, 4]. However, the storage medium and temperature may affect biomechanical evaluation *ex vivo*, which has been demonstrated with other tissues such as the cornea [5]. The changes in lenticular biomechanical properties can be an important biomarker for lens tissue integrity during lengthy *ex vivo* studies.

The majority of lens biomechanical assessments have been performed with mechanical testing, which is invasive and can be destructive. Thus, there is a need for noninvasive biomechanical measurement techniques in order to perform longitudinal investigations. Ultrasound elastography (USE) [6, 7]and magnetic resonance elastography (MRE) [8] are clinically-available techniques that have been used to evaluate the biomechanical properties of the lens. Although these techniques have provided useful insights into lenticular biomechanical properties, their relatively poor spatial resolution and contrast, and need for contact-based excitation may not be appropriate for thorough investigations of lenticular biomechanical properties. Here, we performed serial measurements up to 24 hours on extracted porcine lenses with a noninvasive elastographic technique, optical coherence elastography (OCE). OCE has micrometer-scale spatial resolution and sub-nanometer levels of displacement sensitivity with phase-resolved detection [9-11], which is particularly useful for ensuring integrity of the lens because detectable displacements only require very small forces. Thus, OCE has been used previously to measure the biomechanical properties of the lens *in situ* [4, 12].

In this work, OCE was used 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 in M-199 medium. Our results show that that lower incubation temperatures or higher pH of medium increase stiffness of the lens.

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2. MATERIALS AND METHODS

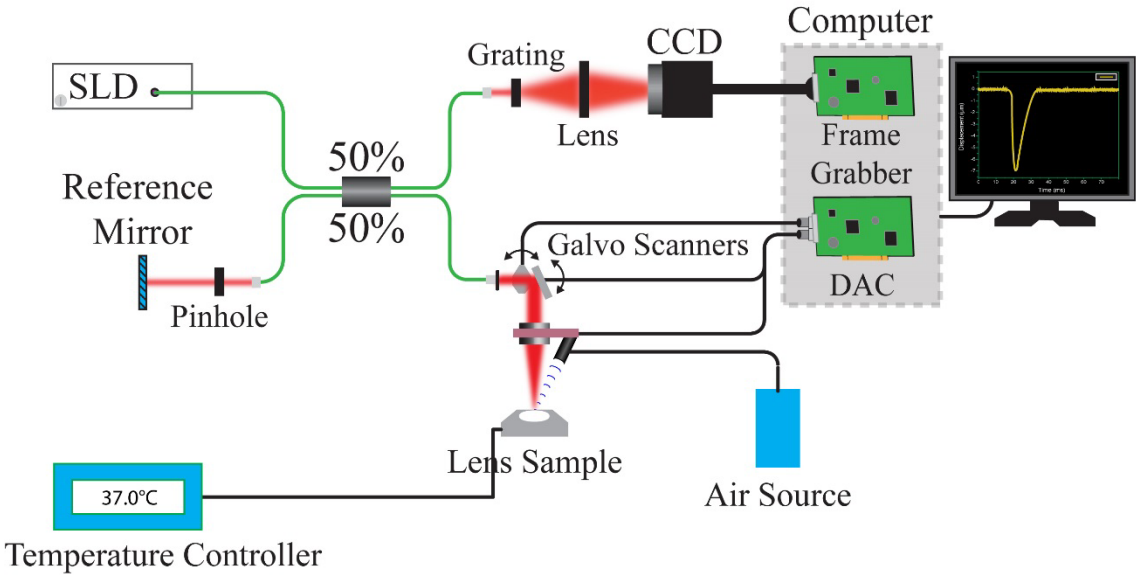

Figure 1. Schematic of the experimental setup. CCD – charge-coupled device; DAC – digital to analog converter; SLD – superluminescent diode.

The OCE system was based on a home-built spectral domain OCT (SDOCT), an air-pulse excitation system, and thermal controller for the lenses [13, 14]. A schematic of the system is shown in Fig. 1. The SDOCT system utilized a superluminescent diode (SLD) with a central wavelength of 840 nm and bandwidth of 49 nm. The displacement stability of the system was 12 nm in air as measured from the surface of a sample lens. The air-pulse delivery system used an electronically controlled pneumatic solenoid and control unit to produce a short duration (≤1 ms) air-pulse that was synchronized with the SDOCT system [15]. The air pressure was controlled by a pneumatic valve and monitored by a pressure gauge. The air-pulse was targeted at the apex of the lens. Lenses (n=30) were removed from fresh porcine eye-globes (Sioux-Preme Packing Co. IA, USA). The lenses were separated into three different experiments. The first set of measurements was focused on assessing the effects of the storage medium (PBS, DMEM, and M-199) on lenticular stiffness (N=3 for each medium). The lenses were separated into three groups. Each group were placed into PBS, DMEM, and M-199 medium and incubated for 24 hours at temperature 37°C, and pH=7.0. The effect of incubation temperature on lens stiffness was measured in the second set of experiments. The lenses were separated into three groups and placed into M-199 medium (Sigma-Aldrich Co., MO, USA), incubated for 12 hours in 4°C, 22°C and 37°C at pH=7.0 (N=3 for each temperature). The effects of pH on the lenticular stiffness were investigated on the third group of lenses. The lenses were separated into four groups and placed in M-199 medium and incubated for 24 hours in 37°C at pH=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 solutions were used to adjust the pH to the target value. The short duration air-pulse induced small amplitude displacements (≤10 μm) on the surface of lens that propagated as an elastic wave. Successive M-mode images ($n = 251$) were acquired over a ~6.1 mm line [15], where the center of the scan and air-pulse 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 [16]. 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 [17], v=0.5 was Poisson's ratio [18], and c_g was the OCE-measured elastic wave group velocity.

3. RESULTS

Figure 2 shows the results of the estimated Young's modulus of the lenses after storage in different media. The estimated Young's modulus of the lenses that were incubated in PBS showed a significant increase over 18 hours, and it increased 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 compared to 18 hours of incubation in PBS. After 12 hours, the lenses incubated in DMEM showed increase in stiffness from 6.1±0.6 kPa to 8.3±0.2 kPa. However, there was a dramatic increase in stiffness from 6.1±0.6 kPa at 0 hours to 13.7±1.8 kPa and 11.6±2.9 kPa after 18 and 24 hours of incubation, respectively. However, the lenses incubated in M-199 showed decrease in stiffness 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 preserves the biomechanical properties of excised porcine lenses better than DMEM or PBS at 37°C over a period of 24 hours.

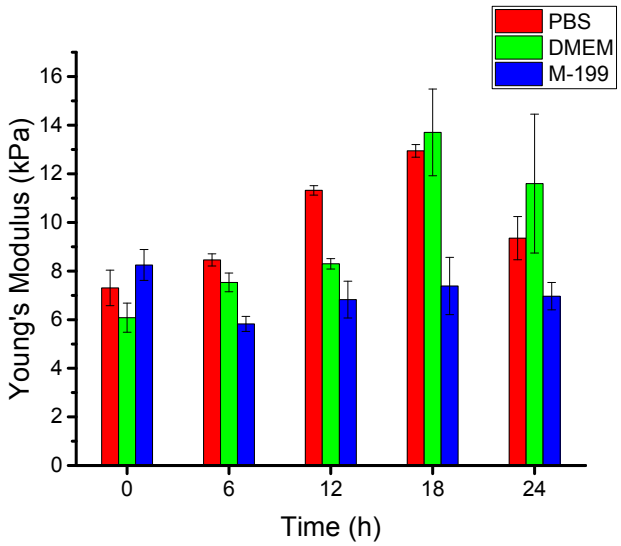

Figure 2. The estimated Young's modulus of the lenses incubated in the different indicated media over 24 hours (N=3 for each medium).

As Fig. 3 shows, 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 increased from 6.0±0.4 kPa to 6.8±0.4 kPa after 12 hours. After 12 hours, the Young's modulus of the lenses incubated at 37°C decreased from 5.5±0.2 kPa to 4.4±0.4 kPa. The results showed that while the stiffness of the lenses increased when incubated for 12 hours at 4°C and 22°C, the lenticular elasticity decreased slightly when the lenses were incubated for 12 hours at 37°C.

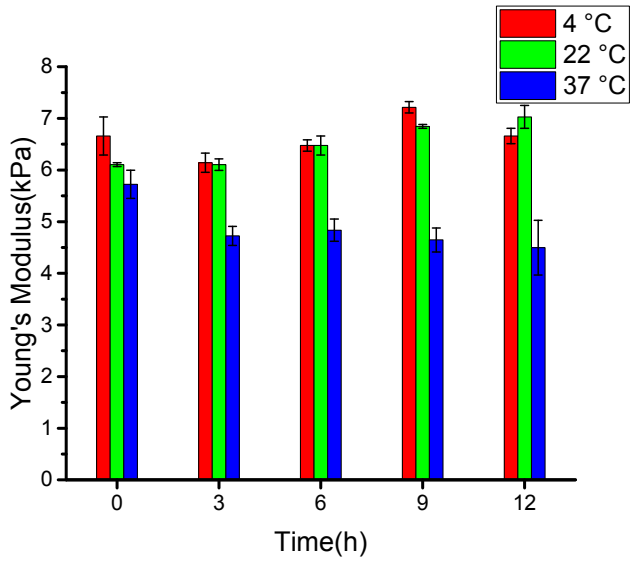

Figure 3. The estimated Young's modulus of the lenses incubated in M-199 medium at 4°C, 22°C, and 37°C over 12 hours (N=3 for each temperature).

Figure 4 plots the results of the estimated Young's modulus of the porcine lenses incubated in M-199 medium at different pHs (4.0, 5.0, 6.0 and 7.0). The Young's modulus of the lens in pH=7.0 medium decreased slightly from 7.4±0.8 kPa to 5.7±1.5 kPa. However, as the pH value decreased, the stiffness of the lenses increased, indicating damage to the lenses. The most dramatic change in elasticity occurred when the lenses were stored at a pH of 4.0, where the stiffness increased from 7.1±0.3 at 0 hours to 12.0±1.3 after 12 hours.

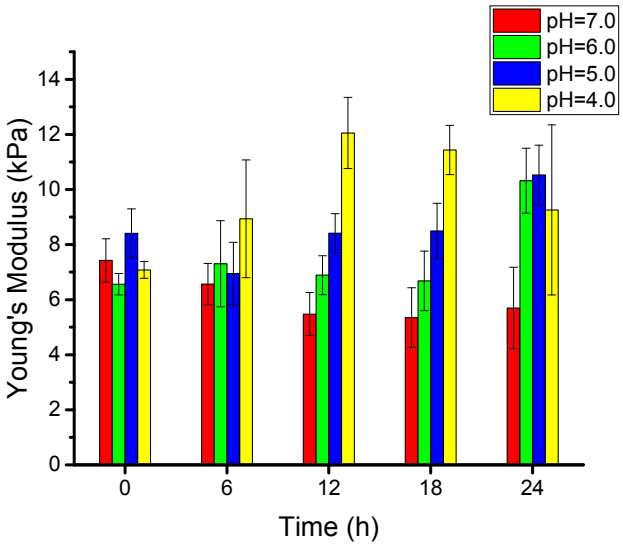

Figure 4. The estimated Young's modulus of the lenses incubated in M-199 media at various pHs over 24 hours (N=3 for each pH).

4. CONCLUSIONS AND FUTURE WORK

In this study, we evaluated the changes of *ex vivo* porcine lenticular biomechanical properties after they were incubated in different media, temperatures, and pHs for up to 24 hours. The results showed that OCE could be used to assess lenticular biomechanical properties and might be useful for detecting and, potentially, characterizing lenticular integrity.

M-199 medium, 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 showed a decrease in elasticity, but the lenses incubated at 4°C and 22°C showed an increase in stiffness, indicating possible structural changes in the lens. 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 future work will entail utilizing a more robust analytical model to obtain quantitative biomechanical parameters such as viscoelasticity as well as evaluating the effects of lenticular biomechanical properties while the lens is within the eye-globe.

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