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● *Original Contribution*

THE MECHANICAL PROPERTIES OF *EX VIVO* BOVINE AND PORCINE CRYSTALLINE LENSES: AGE-RELATED CHANGES AND LOCATION-DEPENDENT VARIATIONS

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Abstract—The mechanical properties of *ex vivo* animal lenses from three groups were evaluated: old bovine (25–30 mo old, $n = 4$), young bovine (6 mo old, $n = 4$) and young porcine (6 mo old, $n = 4$) eye globes. We measured the dynamics of laser-induced microbubbles created at different locations within the crystalline lenses. An impulsive acoustic radiation force was applied to the microbubble, and the microbubble displacements were measured using a custom-built high pulse repetition frequency ultrasound system. Based on the measured dynamics of the microbubbles, Young's moduli of bovine and porcine lens tissue in the vicinity of the microbubbles were reconstructed. Age-related changes and location-dependent variations in the Young's modulus of the lenses were observed. Near the center, the old bovine lenses had a Young's modulus approximately fivefold higher than that of young bovine and porcine lenses. The gradient of Young's modulus with respect to radial distance was observed in the lenses from three groups. (E-mail: emelian@mail.utexas.edu) © 2013 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Crystalline lens, Young's modulus, Shear viscosity, Laser-induced microbubble, Acoustic radiation force, Ultrasound measurement, Presbyopia.**

INTRODUCTION

When a near object is in focus, the eye accommodates; the crystalline lens becomes thicker and rounder in configuration for normal human eyes (Drexler et al. 1997; Strenk et al. 2004, 2005). Accommodation power degrades with age, resulting in presbyopia (Bailey et al. 2010; Beers and Van der Heijde 1994; Weale 2000). There is strong evidence that age-related changes in the mechanical properties of the lens play a major role in the development of presbyopia (Charman 2008). To evaluate further the processes that result in accommodation changes and presbyopia development, a comprehensive understanding of the viscoelastic properties of the crystalline lens is required.

To characterize the mechanical properties of the crystalline lens, different techniques have been developed (Assia et al. 1997; Baradia et al. 2010; Burd et al. 2011; Fisher 1971, 1973; Heys et al. 2004; Heyworth et al. 1993; Kikkawa and Sato 1963; Pau and Kranz 1991; Sharma et al. 2011; Soergel et al. 1999; Tabandeh et al. 2000; van Alphen and Graebel 1991; Weeber et al. 2005, 2007). Researchers reported that the stiffness of the lens as a whole object and variations of lens stiffness with respect to equatorial distance increase with age. Dynamic mechanical analysis has shown that in human lenses older than 50 years, stiffness of the nucleus is an order of magnitude greater than that of the cortex (Heys et al. 2004; Weeber et al. 2007). Furthermore, between the ages of 14 and 78 year old, an almost 1000-fold increase in stiffness of the lens nucleus was reported (Heys et al. 2004). However, most of the current techniques used to measure the lens elasticity are based on direct mechanical testing and usually can be used only *in vitro*.

To assess the mechanical properties of a crystalline lens *in vivo*, microbubble-based acoustic radiation force technique can be used (Aglyamov et al. 2007; Emelianov et al. 2004; Erpelding et al. 2005, 2007a, 2007b; Hollman et al. 2007; Ilinskii et al. 2005; Milas et al. 2003a, 2003b). In this technique, acoustic radiation force is applied to a microbubble created by

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laser-induced optical breakdown in the lens. The displacement of the microbubble is measured by ultrasound and used to evaluate lens elasticity. Compared with other methods, this non-invasive approach does not require contact with the crystalline lens; therefore, the intact lens can maintain its intrinsic mechanical properties during the measurements. In addition, remote assessments of the local viscoelastic properties of the crystalline lenses can also be achieved. The mechanical property changes in both porcine and human crystalline lenses regarding age and locations in lenses were investigated using a microbubble-based technique (Erpelding et al. 2007b; Hollman et al. 2007). In these studies, the local elastic properties were inversely proportional to the maximum displacement of the microbubble; however, the maximum displacement depends on the magnitude of acoustic radiation force. Therefore, to measure the elastic properties of surrounding tissue accurately, the magnitude of acoustic radiation force on the microbubble surface should be evaluated. However, estimation of the magnitude of acoustic radiation force is a difficult task given the unknown attenuation of sound in surrounding tissue. To overcome this limitation, we have previously proposed the use of the temporal characteristics of the dynamics of the microbubble (Aglyamov et al. 2007, 2012; Ilinskii et al. 2005; Karpiouk et al. 2009; Yoon et al. 2011). The temporal response of tissue interrogated by an acoustic radiation force depends on the mechanical properties of the tissue (Sarvazyan et al. 1998). We have demonstrated that the time of maximum displacement of the microbubble under impulsive acoustic radiation pressure is invariant, although the magnitude of acoustic radiation force changes. Therefore, the magnitude of acoustic radiation force becomes just a scaling factor that determines the maximal displacement of a microbubble.

In our previous work, the mechanical properties of gelatin phantoms were estimated from the temporal characteristics of the motion of rounded objects within gelatin and were found to be in good agreement with direct measurements (Aglyamov et al. 2007, 2012; Ilinskii et al. 2005; Karpiouk et al. 2009; Yoon et al. 2011). To increase both the sensitivity and the accuracy of the measurement by using impulsive acoustic radiation force, a high pulse repetition frequency (PRF) ultrasound system has been introduced (Yoon et al. 2012). We have validated this high PRF system successfully using *ex vivo* bovine crystalline lenses and comparing the obtained data with the results of independent mechanical tests (Yoon et al. 2012).

In this study, we investigate age-related changes and location-dependent variations of the mechanical properties of bovine and porcine crystalline lenses. We generated the laser-induced microbubbles at various locations in the crystalline lenses by focusing a single nanosecond laser pulse. Next, the dynamic behavior of the laser-induced microbubbles, displaced by an impulsive acoustic radiation force, was measured using the high PRF ultrasound system. We reconstructed the local Young's modulus of lens tissue by measuring the dynamics of the laser-induced microbubble and comparing it with the theoretically calculated values. We compared the viscoelastic properties of young and old bovine lenses to explore age-related changes. In addition, location-dependent variations of the mechanical properties were measured using bovine and young porcine lenses.

MATERIALS AND METHODS

Lens preparation

All tissue samples were obtained from Sierra for Medical Science, Inc. (Whittier, CA, USA). The eye globes were shipped overnight in a thermally insulated box with ice packs. Three groups of tissue samples were used: old bovine (25–30 mo old), young bovine (6 mo old) and young porcine (6 mo old) eye globes. In each group, four crystalline lenses (n = 4, samples 1–4), excised from the eye globes, were used in the experiments. All the experiments were performed within 12 h after the tissue samples arrived at our facility.

The lens was extracted carefully from an eye globe, and the lens capsule was removed by making small tears at the lens equator. The lens was placed and secured in a lens holder filled with 5 mL of 6% gelatin solution. The anterior of the lens faced the bottom of the lens holder. During the experiments, the lens and the lens holder were kept in phosphate-buffered saline (PBS; Sigma-Aldrich, Inc., St Louis, MO, USA) to minimize changes in the mechanical properties of the lens.

Figure 1a presents a coordinate system defined within the lens. Lenses from old bovine, young bovine and young porcine eye globes had diameters (denoted by D in Fig. 1a) of 16 ± 0.5 mm, 12 ± 0.3 mm and 10 ± 0.5 mm, respectively. Total thicknesses (denoted by h in Fig. 1a) from the anterior to the posterior part were 11 ± 0.6 mm, 9.0 ± 0.3 mm and 7.0 ± 0.3 mm for old bovine, young bovine and young porcine lenses, respectively.

Laser-induced microbubble generation

Laser-induced microbubbles with typical radii of 45–60 μm were generated along the S-axis (Fig. 1a). No correlation was observed between the sizes of microbubbles and their locations within the lens. The typical lifetime of the bubbles was 40 min to 1 h. Using the 3-D translational stage attached to the lens holder with the crystalline lens inside, the distance d between the S-axis and anterior part (denoted by d in Fig. 1a) was set to 4, 4 and 3 mm in experiments with old bovine,