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Spatial Variations of Viscoelastic Properties of Porcine Vitreous Humors

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Abstract

Using a microbubble-based acoustic radiation force approach, spatial variations of Young's modulus and shear viscosity of the porcine vitreous humors in two groups— young pigs (6 months old) and mature pigs (2 to 3 years old)— were measured *in situ*. The measurements in these groups (4 specimens in each group) were performed in several positions along an anterior-to-posterior direction. At each position, microbubbles were generated by focusing a nanosecond pulsed laser beam and the displacement of each microbubble in response to an impulsive acoustic radiation force was measured every 10 μ s using a custom-made high-pulse-repetition-frequency ultrasound system. Based on measured dynamics of the microbubble, Young's modulus and shear viscosity at various locations of the vitreous were reconstructed. Young's moduli of the young and mature porcine vitreous at anterior region were the highest, whereas the central region had the lowest values, indicating the clear spatial variations in the vitreous humor elasticity in both groups.

I. Introduction

Changes in the viscoelastic properties of the vitreous humor are an indicator of the physiological and pathological changes in the eye [1]–[3]. Vitreous liquefaction is caused by the collapse of the internal network of collagen fibrils and hyaluronic acid [4], [5] resulting from aging or pathological reasons [5]–[7], and may lead to posterior vitreous detachment (PVD) [8], [9]. This is a natural process as age advances; however, in some cases of PVD, if the detached posterior vitreous maintains attachment to the retina, the risk of retinal tears and detachment occurs, which is directly related to blindness [3], [6], [7]. Therefore, monitoring the changes of the mechanical properties of the vitreous can diagnose the possible onset of retinal detachment.

Various techniques have been developed to measure the mechanical properties of the vitreous humor: nuclear magnetic resonance imaging for the liquefaction of the vitreous [10]; dynamic mechanical measurements with compression [11]; the relaxation of the scattering pattern in the human eye [12]; radiation force [13]; bulk measurements using cleated tools [14]; cavitation rheology [15]; and ultrasound image velocimetry [16]. Researchers qualitatively measured phenomena related to physiological changes in the eyes [10]–[12]. Using 25-mm-diameter cleated tools, the mechanical properties of the bulk vitreous was measured quantitatively [14]. The shear storage moduli of the porcine vitreous ranged from 2.8 to 10 Pa [14]. Most recently, *in vivo* measurements of the mechanical properties of the vitreous humor were performed using the relationship between the applied pressure and the size of cavitation bubbles at the needle tip [15]. The results showed that the storage moduli of bovine vitreous were between 120 and 660 Pa [15]. A suggested possible explanation of the difference between values obtained in the literature [14], [15] is that the elastic modulus of the vitreous humor decreased monotonically after the vitreous was removed from the eye.

A microbubble-based acoustic radiation force technique to remotely measure the localized viscoelastic properties of soft tissues has been previously introduced [17]–[21]. Once the laser-induced microbubble is created by optical breakdown of a focused laser beam, the acoustic radiation force is applied to the microbubble to produce a dynamic response, which is directly related to the viscoelastic properties of the tissue. We advanced the microbubble-based acoustic radiation force technique by combining the theoretical model and experimental measurements [22]–[29]. Importantly, the dynamic approach does not require the knowledge of the acoustic radiation force magnitude to reconstruct viscoelastic properties of a medium [24], [30]. We have also developed the high-pulse-repetition-frequency (PRF) ultrasound system which improved the accuracy of the microbubble displacement measurements [31]. In our previous work, we validated our theoretical model [23] of the dynamics of rounded objects such as solid spheres [24], [26] and laser-induced microbubbles [30] under acoustic radiation force in a viscoelastic medium. The approach has been implemented using a high-PRF ultrasound system and applied successfully in the measurement of the viscoelastic properties of animal crystalline lenses of different ages and species [31], [32].

In this paper, we demonstrated that this technique is also able to estimate the local viscoelastic properties of the vitreous remotely. The vitreous was not removed from the eye to keep the *in situ* state as much as possible. Moreover, we investigated the spatial variations and age-related changes of the mechanical properties of the porcine vitreous. We generated microbubbles along an anterior-to-posterior direction in the vitreous by focusing nanosecond laser pulses. The displacements of the microbubbles were measured by a custom-built high-PRF ultrasound system [31]. Reconstruction of the viscoelastic properties of the vitreous was performed by comparing microbubble displacements obtained by experimental measurements to theoretical calculations [30]–[32].

II. Materials and Methods

A. Vitreous Humor Preparation

Porcine eye globes were obtained from Sierra for Medical Science Inc. (Whittier, CA). The whole eyes were shipped over night in a thermo-insulated box with ice packs. Two groups of the vitreous specimens were prepared: young (6 months old) and mature (2 to 3 years old) porcine eyes. Four vitreous humors (specimens 1 to 4, S1 to S4) in each group were used. All the experiments were done within 10 h of the specimens arriving at our facility. Eyes were slowly warmed up to room temperature, at which all the experiments were performed.

For the laser-induced microbubble experiments, three areas of sclera denoted by R1, R2, and R3 in Fig. 1(a) were removed with a scalpel and tweezers to allow focusing of a pulsed laser beam and creating a laser-induced microbubble (R1), to deliver ultrasound beams for displacement measurements of the microbubble (R2), and to allow optical measurements of microbubble size (R3).

The R1–R3 areas of the sclera were removed very carefully to minimize any possible changes of vitreous humor. The microbubbles were created far away from the access areas, as shown in Fig. 1, and all experiments were performed as soon as possible. When the access areas were created, the eyeballs were immediately placed into a phosphate-buffered saline (Sigma-Aldrich Inc., St Louis, MO) to prevent changes of the specimen.

B. Generation of Microbubbles in the Vitreous Humor

Laser-induced microbubbles were generated along the x -axis (Fig. 1). Because eyes from mature pigs were larger than those from young pigs, the spacing of measurement points was set proportional to eye dimensions. The sizes of the vitreous in the anterior-to-posterior direction were measured as distances from the posterior lens capsule surface of the crystalline lens to the vitreoretinal interface. These distances were about 11 and 16 mm for young and mature vitreous, respectively. The first location of the microbubble [P1 in Figs. 1(b) and 1(c)] was 500 μm away from the posterior apex of the crystalline lens in all cases. In the smaller young vitreous, microbubble locations were spaced at 2.3-mm intervals, covering a total range of approximately 7 mm. In the bigger mature vitreous, microbubble locations were spaced at 3.3-mm intervals, covering a total range of approximately 10 mm. Thus, the ratio of the distance between adjacent microbubbles to the size of the vitreous was kept constant (about 0.21) for proper comparison of the viscoelastic properties of the vitreous humors from young and mature pigs.

We generated laser-induced microbubbles inside the vitreous humor by using a pulsed Nd:YAG laser (Polaris II, New Wave Research, Fremont, CA) operated at $\lambda = 532\text{nm}$. The pulse duration was 5 ns, and energy per pulse was 8 mJ. The custom-built objective lens had a numerical aperture of 1.13 and a working distance of 8.0 mm. By focusing the laser beam with the objective lens, we could generate microbubbles at the desired location (P1 to P4 in Fig. 1) inside the vitreous [31]. The size of the microbubble was monitored by an optical microscope (AM411T, Dino-Lite, Torrance, CA) operating at 230 $\times$ magnification. Generated microbubbles stayed at the same location for about two hours and then disappeared. The sizes of microbubbles were almost constant during the experiments—the

microbubbles were stable for about two hours and the measurements for one microbubble took less than 20 min. Microbubble radii ranged approximately from 20 to 75 μm . The measurements were repeated three times. The first set of measurements started from the anterior position (P1) and ended at the posterior position (P4). The second set of measurements was done in the reverse order. The last set of measurements used the same order as the first one. These three measurements were averaged to account for the position-dependent bias that might occur. Based on three measurements of the microbubble radius, the mean and standard deviation values were determined.

C. Experimental System and Signal Processing

Prior to the experiments, to avoid the distortion of the vitreous and to maintain its shape and orientation, we made a 6 wt% gelatin base that mimicked the shape of the eye. During the microbubble experiments, the previously prepared eye globe with three open areas (Fig. 1) was placed on the gelatin base as shown in Fig. 2. Both the vitreous in the eye globe and the gelatin base were in a holder (Fig. 2) and the whole holder was kept in phosphate-buffered saline to minimize changes to the viscoelastic properties of the specimen and to deliver ultrasound radiation (Fig. 2). The holder was attached to a 3-D translational stage and moved to generate laser-induced microbubbles at different locations (P1 to P4 in Fig. 1) in the vitreous.

An ultrasound excitation transducer with a center frequency of 3.7-MHz, f-number of 2, and bandwidth of 17% (Valpey Fisher, Hopkinton, MA) was located at the top of the cuvette and used to produce acoustic radiation pressure and to induce translational motion of the microbubble. Two ultrasound transducers (denoted T and R in Fig. 2) with center frequencies of 25 MHz, f-number of 4, and bandwidths of about 50% (Olympus NDT, Waltham, MA) were located on both sides of the excitation transducer. These transducers were used to transmit/receive ultrasound pulses and to track the displacement of the microbubble. By separating ultrasound transducers T (transmit) and R (receive), the desired high pulse repetition frequency (PRF = 100 kHz) was achieved [31]. The diameters of the foci of ultrasound transducers T and R were about 200 μm . The foci of all three transducers were aligned at the location of the microbubble. The axes of all three transducers were on three edges of a tetrahedron pointing at a common vertex, which was the location of the microbubble. The angles between the three ultrasound transducers were 35° . The measured displacements of microbubble were cosine corrected by considering the angles between three ultrasound transducers [23].

As shown in Fig. 2, the main control was used to manipulate the pulse-echo sequence and acoustic radiation pulse generation. For the pulse-echo sequencing, a train of single-period sinusoidal pulses with center frequency of 25 MHz, PRF of 100 kHz, and the total duration of 18 ms was used. For the acoustic radiation pulse generation, a top-hat pulse (center frequency = 3.7 MHz) with a duration of 2.4 μs was triggered at 60 μs after the first pulse was generated. Pulses for pulse-echo sequence and acoustic radiation pulse generation were amplified by RF power amplifiers A (gain = 55 dB; model A150, E&I, Rochester, NY) and B (gain = 50 dB, model 2100L, ENI, Rochester, NY), respectively. The backscattered ultrasound echoes were acquired by the ultrasound transducer R and amplified by the

ultrasound receiver (DPR 300 pulser/receiver, JSR Ultrasonics, Pittsford, NY). Both RF signals of the train of single-period pulses from the main control and of the amplified ultrasound echoes were saved using two channels of the 12-bit analog-to-digital data acquisition card (CompuScope 12400, GaGe Inc., Montreal, Canada) operated at 200 MHz sampling frequency. Therefore, RF raw data were comprised of the train of short pulses and backscattered ultrasound echoes. When a laser-induced microbubble was created and the pulse-echo started working, the arrival time of the first echo defined the initial location of the microbubble and changes in the arrival times of the following echoes determined the microbubble displacement. These displacements were found using a cross-correlation speckle tracking method [31], [33]. The kernel size and search window for cross-correlation tracking were 94 and 188 μm , respectively.

Reconstruction of the viscoelastic properties of the porcine vitreous humors using the microbubble approach was performed by finding the best fit between microbubble displacement profiles measured in the experiments and calculated by theory [23], [24], [26], [30]–[32]. The measurements of the microbubble's displacements were repeated three times using a single microbubble at each location. The time of maximum displacement of a microbubble (t_{max}) does not change with different magnitudes of acoustic radiation force (F_0) applied to the microbubble, whereas the maximum displacement of a microbubble (U_{max}) does change. Thus, Young's modulus (E) and shear viscosity (η) were found by measuring t_{max} and the decaying profiles of the microbubble. Experimentally measured t_{max} was matched to theoretically calculated t_{max} by varying E . The decaying profiles of a microbubble displacement obtained from experimental measurements and theoretical calculations were compared by varying η . Once the best fit between the theory and the experiments was reached, the estimation of E and η of a tissue specimen was complete. The detailed procedure of the estimation of E and η is described elsewhere [24], [26], [29]–[31].

D. Statistical Analysis

First, to investigate location-dependent variations of the mechanical properties at different locations (P1 to P4 in Fig. 1) inside each specimen from two groups [young ($n = 4$) and mature ($n = 4$) porcine eyes], statistical significance was calculated by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) *post hoc* test. Values were considered as significant if $p < 0.05$ for both ANOVA and HSD tests. Next, Wilcoxon–Mann–Whitney test was used to detect age-related changes of the mechanical properties of the vitreous between two groups, and $p < 0.05$ was accepted to be statistically significant.

III. Results

Examples of displacements of microbubbles (independently measured three times and averaged) in anterior (thin-dashed line, P1) and central (thick-solid line, P3) regions of the vitreous are presented in Fig. 3. Two microbubbles with radii of $33 \pm 5 \mu\text{m}$ and $32 \pm 2 \mu\text{m}$ (mean and standard deviation) were created in the young porcine vitreous humor at positions P1 and P3, respectively. Theoretically obtained microbubble displacements are presented in Fig. 3. To reconstruct the viscoelastic properties of the local areas around P1 and P3, the

scaling factors of acoustic radiation force (F_0) were chosen as 180 and 75 mN, respectively. The values of F_0 were not the same because the actual acoustic radiation force delivered to the microbubble depends on several parameters such as ultrasound attenuation, shape of the surface, and the focusing of the excitation transducer. Note that the reconstruction procedure does not depend on the scaling factor F_0 [23], [24], [30]. Young's moduli in the anterior (P1) and central (P3) regions were reconstructed to be 34.0 ± 1.3 Pa and 8.3 ± 0.2 Pa, respectively. After matching $t_{\max}$ between measurements and theory (Fig. 3), the shear viscosity was also estimated by comparing the decaying profiles of the displacements obtained from theory and experiments. The shear viscosities were found to be 0.052 ± 0.003 Pa·s (P1, anterior part) and 0.035 ± 0.005 Pa·s (P3, central part), respectively.

Fig. 3 demonstrates how dynamics of a microbubble in response to acoustic radiation force depends on the elastic properties of surrounding medium. The $t_{\max}$ of microbubble in the soft central region (P3, solid lines in Fig. 3) is longer than that in the stiff anterior region (P1, dashed lines in Fig. 3). This observation corresponds to the theoretical model, which predicts similar behavior of the microbubbles in stiff and soft viscoelastic media. Although, as expected, the maximum displacement of the microbubble ($U_{\max}$) in the central region (P3) is larger than that in the anterior region (P1) (Fig. 3), the ratio of displacement amplitudes does not correspond to the ratio of Young's moduli in these two locations because of difference in the magnitude of acoustic radiation force on the microbubbles. However, only the temporal characteristics of the microbubble's displacement are used to assess viscoelastic properties, whereas the radiation force magnitude does not affect the reconstruction results [23], [24], [30].

Young's modulus measurement results are shown in Table I as a mean value and one standard deviation (SD in Table I) obtained from three measurements performed using same laser-induced microbubble at each location. Anterior (P1), central (P2 and P3), and posterior (P4) locations of each vitreous specimen from both young and mature groups have statistically significant different mean values of Young's modulus according to one-way ANOVA test ($p < 0.05$). Tukey's HSD *post hoc* test, followed by ANOVA, indicates that each location (P1 to P4) has a mean significantly different from each other ($p < 0.05$) except center locations (P2 and P3) in specimen 4 from young (denoted as * in Table I) and specimen 1 from mature (denoted as ** in Table I) pig groups. Detailed examples of the displacements of microbubbles at anterior location (P1) and central location (P3) in the second specimen of young porcine vitreous were already presented in Fig. 3. Results of the measurements at some locations are not available because of misalignment among equipment (NA in Table I). The anterior region [P1 in Fig. 1(b)] is the stiffest and the central region is the softest in both young and mature specimens.

Next, we sought to determine age-related changes in the Young's modulus between two different age groups (6 months old versus 2 to 3 years old). The Young's moduli of two age groups at central locations (P2 and P3) are not statistically significantly different ($p = 0.1651$ and 0.7222 obtained by Wilcoxon–Mann–Whitney test in Table I). Statistical significances at the anterior and the posterior locations (P1 and P4) of the vitreous humors are not easy to decide because p values are similar to 0.05 (denoted as # in Table I). Therefore, these data

suggest that Young's moduli of the vitreous along the anterior-to-posterior direction between two different age groups do not have strong statistical significance.

The shear viscosity was estimated using the same RF data as the Young's modulus estimation (Fig. 4). The means of the shear viscosity vary from 0.022 ± 0.008 Pa·s to 0.053 ± 0.0058 Pa·s for the young vitreous humor and from 0.0117 ± 0.0029 Pa·s to 0.043 ± 0.0058 Pa·s for the mature vitreous humor. One-way ANOVA test was performed on the measurements of shear viscosity values at all locations (P1 to P4) in each vitreous specimen from young and mature pigs to investigate the spatial variations in the vitreous. Obtained p values are shown in the legends in Figs. 4(a) and 4(b). Tukey's *post hoc* test, followed by ANOVA, indicates that all specimens from young pigs have statistically significant difference in shear viscosity values ($p < 0.05$); however, that difference for mature pigs is not significant except specimen 1. Therefore, the spatial variation of the shear viscosity is not as much distinct as that of Young's modulus of the vitreous, particularly for mature pigs.

To investigate age-related changes of shear viscosity between two groups, Wilcoxon–Mann–Whitney test was used and there are no significant differences between two age groups in central regions (P2 and P3). However, anterior (P1) and posterior (P2) locations of the vitreous have significantly different shear viscosity values between two age groups. P values at P1, P2, P3, and P4 between young and mature groups are less than 0.001, 0.0836, 0.8286, and less than 0.001, respectively. The age-related changes of the shear viscosity are observed in anterior and posterior regions, but not in the central region of the vitreous.

IV. Discussion

We measured the viscoelastic properties of the vitreous humors of young (6 months old) and mature (2 to 3 years old) pigs using laser-induced microbubbles interrogated by acoustic radiation force. Although not ideal, we chose porcine vitreous humors as a surrogate of human vitreous after considering several characteristics of the vitreous such as volume and collagen, hyaluronic acid, and protein content [34]. By using a microbubble-based acoustic radiation force approach, we characterized the spatial variations of the mechanical properties of the vitreous. In addition, we also compared the changes in the mechanical properties of the vitreous with age, but the age-related changes of the viscoelastic properties were much less significant than location-dependent variations. One possible explanation of this finding is that most lower mammals have relatively small changes in the vitreous body as age advances [34]. Furthermore, given the pig's lifespan of 10 to 15 years in average, the considered age difference between two groups may be insufficient to exhibit significant changes in both shear elasticity and viscosity. Therefore, more experiments with wider age variations and larger numbers of specimens are required to confirm this finding.

Detection of liquefaction in the vitreous requires exact information about local viscoelastic properties because the aggregation of collagen fibrils starts locally by making a liquid pocket in the vitreous [2]. However, the traditional rheometers only provide the averaged information about bulk viscoelastic properties of a specimen. Moreover, wall-slip condition hampers the accuracy of measurements in a parallel-plate testing in rheology [35]. Thus, the advantage of the proposed microbubble-based acoustic radiation force technique over the

conventional rheometers is the ability to measure the localized viscoelastic properties of the vitreous accurately.

In addition to elasticity, the shear viscosity of the vitreous can also be evaluated based on the measured dynamics of the microbubble. Even though the shear viscosity (Fig. 4) has less significant spatial variations compared with the Young's modulus (Table I), spatial variations in the vitreous from young pigs are quite statistically significant, presenting similar tendency compared with spatial variations of Young's modulus. In other words, the anterior region of the vitreous has a higher shear viscosity than the posterior and central regions, whereas the lowest values exist in the central region.

To secure the measurement locations [P1 to P4 in Fig. 1(b)], we kept the vitreous inside the sclera together with other ocular parts such as the crystalline lens. Difficulties in vitreous humor experiments are a lack of landmarks to guide the orientation of the vitreous and distortion of the shape of the vitreous if taken out of the eye. We determined the anterior, central, and posterior regions of the vitreous from the locations of other ocular parts, such as the lens [Fig. 1(c)]. Moreover, to prevent the distortion of the vitreous during the experiments, we made a gelatin base that had the same shape as the eye globe so that the shape of the prepared vitreous humor could be maintained. Thus, we could reliably measure the viscoelastic properties of similar locations within the different vitreous humors. All microbubbles were produced far from the boundaries, i.e., at a distance significantly more than bubble diameter, therefore, the change of the boundary conditions by introducing a gelatin base did not affect the microbubble dynamics.

Changes in the mechanical properties of the vitreous with time could affect the results of measurements. Other groups [14], [15] reported the rapid decrease of the Young's modulus in porcine vitreous specimens to a steady state after 20 min of removal from the eye [14]. Such changes were even accelerated if the vitreous membrane was disrupted. In our experiments, to minimize the changes on the mechanical properties, most of the vitreous was kept inside the sclera (Fig. 1) and three relatively small segments of the sclera [R1, R2, and R3 in Fig. 1(b)] were removed to perform the experiment, and the vitreous membranes were not disrupted (i.e., the membrane remained intact). Also, our experiments were completed as quickly as possible. Within 5 min, the eye globe with exposed vitreous humor was prepared and within the next 20 min, 12 measurements (three measurements at each location P1 to P4) were completed. Our results indicate that there is no significant time dependence of the measurements if the vitreous is kept inside the sclera and all experiments are performed quickly.

The mechanical properties of porcine, bovine, and human vitreous humors were reported as ranging from 0.15 to 660 Pa [11], [12], [14], [15]. Some values reported in the literature were extremely low compared with our results [11], [12]. The storage moduli of porcine and bovine vitreous measured with cleated tools [14] and with the cavitation rheology technique [15] were comparable with our results. In our experiments, spatial variations within the vitreous were clearly seen for both young and mature porcine vitreous humors. This result corresponds to the variations in the concentrations of collagen fibrils reported in the literature [11]. Recently, the load-bearing struts (collagen fibrils) and tensile force

(hyaluronan) hypothesis has been suggested to explain the origin of the mechanical integrity of the vitreous [14]. By combining these observations, we can conclude that one possible reason for the spatial variations in the vitreous humors comes from the concentration differences of vitreous components such as collagen fibrils and hyaluronic acid.

V. Conclusions

We applied a microbubble-based acoustic radiation force approach to analyze the mechanical properties of the vitreous humor for the first time. The developed technique measures the viscoelastic properties of the vitreous humors from young and mature pigs locally and accurately. Spatial variations in the Young's modulus of both young and mature porcine vitreous were observed. Age-related dependence of the Young's modulus of the vitreous humors between the two groups was not clearly observed. Young's moduli of the anterior region of the vitreous were higher than those of the central and posterior regions, whereas the central region had the lowest Young's modulus. A similar tendency was found in the shear viscosity of the vitreous from young pigs. The spatial variations of the shear viscosity of mature pig's vitreous were not clearly observed.

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Fig. 1.

Microbubble locations in the porcine vitreous humor. (a) 3-D view of the vitreous humor with three access areas (R1, R2, and R3) in sclera. (b) Side view of vitreous humor. Laser-induced microbubbles were generated along the x -axis; positions from P1 (anterior) through P4 (posterior) are shown. (c) Photograph of a microbubble created next to the crystalline lens at position P1.

Fig. 2.

A schematic view of the experimental setup. The vitreous humor was positioned on a gelatin base that mimicked the shape of the eye globe to prevent the vitreous from collapsing in the holder. A microbubble was produced by a focused laser beam inside the vitreous. An ultrasound excitation transducer (3.7 MHz, dashed arc) was used to generate acoustic radiation force and to induce microbubble displacement. The motion of the microbubble was tracked by two ultrasound transducers T and R (25 MHz, solid arc). The separation of transmit (T) and receive (R) transducers allowed high-pulse-repetition-frequency (PRF) measurements to be achieved. We used 100 kHz PRF for all experiments. The size of the microbubble was monitored by an optical microscope.

Fig. 3.

Displacements of microbubbles positioned in the anterior region (location P1, thin-dashed line) and the central region (location P3, thin-solid line) of young porcine vitreous. A top-hat pulse with a duration of $2.4 \mu\text{s}$, applied at $60 \mu\text{s}$, initiated the microbubble motion. Theoretically calculated displacements of microbubbles in a viscoelastic medium with Young's modulus (E) of 34 Pa (thick-dashed lines, anterior part) and 8.3 Pa (thick-solid lines, central part). In theoretical calculations, the shear viscosity values were $0.052 \text{ Pa}\cdot\text{s}$ (thick-dashed line) and $0.035 \text{ Pa}\cdot\text{s}$ (thick-solid line). Times to reach the maximum displacements of the microbubbles are indicated as t_{max} . The radii of the microbubbles were $33 \pm 5 \mu\text{m}$ (location P1) and $32 \pm 2 \mu\text{m}$ (location P3).

Fig. 4.

The shear viscosity measurements of the vitreous humors from (a) young (6 months old) and (b) mature (2 to 3 years old) pigs. The horizontal axis represents different locations inside the vitreous from P1 (anterior) to P4 (posterior) (Fig. 1) and the vertical axis shows the shear viscosity. P values, obtained by applying an analysis of variance (ANOVA) test on the measurements of the shear viscosity values at all locations in each specimen, are shown in the legends of (a) and (b). Age-related changes of the shear viscosity at anterior and posterior locations (P1 and P4) are observed (p -values obtained by Wilcoxon–Mann–Whitney test from P1 to P4 between young and mature groups are <0.001 , 0.0835, 0.8286, and <0.001 , respectively). Error bars represent $\pm$ one standard deviation.

Young's Modulus Measurements (in Pascals) of Four Vitreous Humor Specimens (S1 to S4) from Young (6 Months Old) and Mature (2 to 3 Years Old) Pigs.

TABLE I

Location		Young pig group (<i>n</i> = 4)				Mature pig group (<i>n</i> = 4)				WMW	
		S1	S2	S3	S4	S1	S2	S3	S4	<i>p</i>	
P1 (AN)	Mean	38.7	34.0 [†]	45.9	28.3	14.9	37.0	29.3	NA		0.0506 [#]
	SD	1.5	1.3	5.4	2.6	1.7	4.3	3.9	NA		
	Mean	NA	11.5	12.5	9.5 [*]	7.0 ^{**}	16.4	16.6	12.0	0.1651	
P2 (CN)	SD	NA	1.0	0.6	1.5	1.7	1.0	0.5	2.0		0.7222
	Mean	NA	8.3 [†]	2.0	9.9 [*]	5.2 ^{**}	9.1	7.8	6.2	0.7222	
	SD	NA	0.2	0.1	0.6	0.2	0.6	0.4	0.2		
P4 (PO)	Mean	11.9	12.5	16.8	16.1	9.1	24.4	24.0	21.7	0.0453 [#]	
	SD	1.6	0.6	0.0	0.8	0.3	0.7	1.1	2.3		
	F	430.0	534.2	142.4	96.6	37.1	84.4	62.3	58.7		
Analysis of variance											
<i>p</i>		<0.05									
		<0.05									

SD = one standard deviation; AN = anterior part; CN = center part; PO = posterior part; WMW = Wilcoxon-Mann-Whitney test to compare young and mature pig groups at each location (P1 to P4); NA= results are not available;

*/** = not statistically significant difference based on Tukey's HSD *post hoc* test, at the significance level of 0.05;

[#] = marginal significance ($p \approx 0.05$) between young and mature pig groups at the designated location;

[†] = examples of detailed dynamics of the microbubbles are shown in Fig. 3.