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Viscoelastic modulus of agarose gels by magnetic resonance elastography using Micro-MRI


* Division of Human Mechanical Systems and Design, Graduate School of Engineering, Hokkaido University
  Kita 13, Nishi 8, Sapporo, Hokkaido 060-8628, Japan
** Division of Human Mechanical Systems and Design, Faculty of Engineering, Hokkaido University
  Kita 13, Nishi 8, Sapporo, Hokkaido 060-8628, Japan
*** Department of Intelligent Machines and System Engineering, Faculty of Science and Technology
  1-bunkyocho, Hirosaki-shi, Aomori-ken 036-8560, Japan
**** Department of Medical Systems Engineering, Division of Artificial Systems Science, Graduate School of Engineering, Chiba University, 1-33, Yayoi-cho, Inage-ku, Chiba-shi, Chiba 263-8522, Japan
***** Department of Mathematics, Faculty of Science, Hokkaido University, Kita 10, Nishi 8, Sapporo, Hokkaido, 060-0810, Japan

Abstract
This study aimed to apply magnetic resonance elastography (MRE) using micro-magnetic resonance imaging (micro-MRI) system for the measurements of viscoelastic modulus in soft matters. The rectangular specimens of 90 × 70 × 50 mm were made of agarose gel with five kinds of stiffness by changing concentrations. The specimens were oscillated with longitudinal waves transmitted by an elastic-bar from a vibration generator in a micro-MRI system. Since the viscoelastic properties depend on the excitation frequency and amplitude, the experimental conditions were selected in the range of 50–250 Hz and 0.1–0.5 mm. The viscoelastic modulus was expressed as storage shear modulus $G'$ and loss shear modulus $G''$. As a result, $G'$ increased with the frequency and amplitude, and the difference of $G'$ between hard and soft gels was obtained. The viscoelastic modulus of agarose gels was measured using the MRE system under the excitation conditions. Furthermore, double-layer specimens composed of 0.6 and 2.0 wt% gels were examined as an application of the MRE system. The difference of wave pattern between the hard and soft parts was observed. The values of $G'$ in the soft parts of the double-layer specimens corresponded to the value of the single-layer specimen, but the values of $G'$ in the hard parts were varied.

Key words: Magnetic resonance elastography, Magnetic resonance imaging, Viscoelasticity, Soft matter stiffness, Elastic modulus

1. Introduction

Magnetic resonance elastography (MRE) (Muthupillai, et al., 1995) is an effective technique for measuring the stiffness of living organs. The measurements of liver (Venkatesh, et al., 2008), lungs (Goss, et al., 2006), breasts (Sinkus, et al., 2005), brain (Kruse, et al., 2008), and muscles (Basford, et al., 2002) have been conducted in vivo using clinical MRI, and the stiffness of tissues associated with diseases has been discussed. In the MRE method, the organs were vibrated to generate transverse waves and the stiffness of the organs was quantitatively calculated from the resulting wave patterns (Muthupillai, et al., 1996). MRE measurements have also been conducted on the stiffness of soft matters and validation of the MRE procedures was discussed (Hamhaber, et al., 2003; Atay, et al., 2008; Mariappan, et al., 2009, 2010; Perreard, et al., 2010; Okamoto, et al., 2011).

Soft tissues and organs have a viscoelastic property. In the literature, Green et al. (2008), Sack et al. (2009), Streitberger et al. (2011), and Clayton, et al. (2011) have measured the viscoelastic modulus of brains in vivo using MRE with clinical MRI, considering both magnitude and phase of the displacement in the tissue. Asbach et al. (2008)
and Klatt et al. (2010) have also measured the viscoelastic modulus of livers in vivo.

The authors developed an MRE system consisting of a micro-magnetic resonance imaging (micro-MRI) with 0.3 T, a bar-type transmitter, and a vibration generator, which allowed the excitation frequency of 50–250 Hz and the amplitude of 0.1–0.5 mm (Tadano, et al., 2012). The bar-type transmitter and the vibration generator were used to generate strong excitation. In that study, it was confirmed that the distribution of displacement was obtained within the agarose gel of 1.2 wt% concentration under the excitation conditions. Furthermore, to decrease the error of viscoelastic modulus, the authors proposed a calculation method for the MRE measurements under the low magnetic field based on the integration of the displacement (Jiang and Nakamura, 2011). It is necessary to apply the MRE instrument and the calculation method to the measurements of viscoelastic modulus of soft matters and to discuss the availability of the system.

Therefore, the present study aimed to investigate the effect of the excitation frequency of 50–250 Hz and the amplitude of 0.1–0.5 mm on the viscoelastic modulus of agarose gels with five kinds of stiffness by using the MRE system. Furthermore, the distributions of the displacement and viscoelastic modulus in the specimens consisting of hard and soft gel parts were investigated as an application of the MRE system. Three kinds of the double-layer specimen were examined in the study.

2. Methods

When a specimen was excited with a sine wave in the MRI, local displacements in the specimen under a gradient magnetic field were calculated from the phase shift of MR signal (Muthupillai, et al., 1996; Manduca, et al., 2001). A complex displacement vector \( \mathbf{u} \) was defined to be composed of displacement at 0 and \( \pi/2 \) of the excitation phase as the real and imaginary parts, respectively (Tadano, et al., 2012). Viscoelastic modulus was described by storage shear modulus \( G' \) and loss shear modulus \( G'' \) as the real and imaginary parts of a complex shear modulus, respectively.

When the specimen is assumed to be incompressive, the force equilibrium in a small region is satisfied with Eq. (1) (Clayton, et al., 2011).

\[
(G' + iG'') \Delta t + \rho(2\pi f)^2 \mathbf{u} = 0
\]  

(1)
The $\rho$ and $f$ indicate the density of the specimen and the excitation frequency, respectively. The conjugate of Eq. (1) is,

$$(G' - iG'')\bar{u} + \rho(2\pi f)^2 \bar{u} = 0$$

(2)

When Eq. (2) is multiplied by $u$ and integrated over the domain size $D$ on the $x$-$y$ plane, it becomes Eq. (3) (Jiang and Nakamura, 2011).

$$G' - iG'' = -\rho(2\pi f)^2 \left( \frac{\int_D|u|^2 \, dxdy}{\int_D u \bar{u} \, dxdy} \right)$$

(3)

The $G'$ and $G''$ are calculated from the following equations.

$$G' = -\rho(2\pi f)^2 \text{Re} \left( \frac{\int_D|u|^2 \, dxdy}{\int_D u \bar{u} \, dxdy} \right)$$

(4)

$$G'' = \rho(2\pi f)^2 \text{Im} \left( \frac{\int_D|u|^2 \, dxdy}{\int_D u \bar{u} \, dxdy} \right)$$

(5)

Figure 1 shows the flowchart of the MRE analysis process. The complex displacement was obtained from the phase shift of MR signal after phase unwrapping. The noise was eliminated from the complex displacement data by using the Lavrent’ev regularization scheme (Xu and Liu, 2010). The viscoelastic moduli were calculated from Eqs. (4) and (5). Then, the distributions of $G'$ and $G''$ were smoothed using the total variation image reconstruction method (Dahl, et al., 2010).

3. Experiments

3.1 Micro-MRI and excitation systems

Figure 2(a) shows an MRE system using a 0.3-T micro-MRI. The system was described in detail by Tadano et al. (2012). The motion sensitizing gradient was controlled according to an MRE sequence based on the spin-echo sequence (TR, 400 ms; TE, 59 ms). The spatial resolution was $1.2 \times 1.2 \times 1.8$ mm. The size of MRI images in horizontal plane including the $x$-$y$ plane was $256 \times 128$ pixels. A bar-shaped transmitter was constructed from an acrylic tip with a diameter of 8 mm and glass fiber-reinforced plastic (GFRP) pipe with outer and inner diameters of 10 and 8 mm, respectively (Fig. 2(b)). The excitation amplitude was confirmed to be equal to the edge displacement of the acrylic tip in contact with the specimen surface.

3.2 Agarose gel specimens

The rectangular specimens of $90 \times 70 \times 50$ mm were made from agarose powder (Kanto Chemical Co., Inc., Japan). The agarose solutions were heated to approximately 100 °C in a plastic case. Agarose gels become harder as the concentration increases. Three specimens of the five stiffness of 0.6, 0.8, 1.2, 1.6, and 2.0 wt% concentrations (type A in Fig. 3) were examined. After the MRE experiments three cylindrical specimens for the compression test of 15 mm in diameter and 10 mm in height were cut out from each specimen. Furthermore, three types of double-layer specimen (n = 3) comprising the softest gel (0.6 wt%) and the hardest gel (2.0 wt%) (type B–D in Fig. 3) were examined. Every specimen was preserved at 5 °C until just before the MRE measurements.

3.3 MRE measurements

The gel specimen was wrapped in a thin plastic film to keep wet and was placed in the MRI gantry on a rubber sheet. Figure 2(b) shows the specimen, the contact position of the vibration-transmitted bar, and the measured plane ($x$-$y$ plane) of the MRE. The $x$-$y$ plane was defined as the half-height of the specimen. A longitudinal excitation was applied to the $x$ direction. The edge of the vibration-transmitted bar was positioned at the center of the $y$-$z$ surface of the specimen and was in continuous contact with the specimen during excitation. The boundary conditions were free, except for the bottom plane, which was fixed by the specimen’s weight. Frequencies of 50, 100, 150, 200, and 250 Hz
with three amplitudes of 0.1, 0.3, and 0.5 mm were selected for this experiments. The measurements were conducted at room temperature (22 ± 2°C). The domain size $D$ and density $\rho$ in the Eqs. (4) and (5) were $6 \times 6 \text{ mm} (5 \times 5 \text{ pixels})$ and $1.0 \text{ g/cm}^3$, respectively. The filtering parameter in the Lavrent’ev regularization scheme was 0.5.

### 3.4 Static compression tests

The static compression tests were conducted using a mechanical testing machine (Instron 4411; Instron Co., Ltd., USA) and a load cell (Static load cell (50 N); Instron Co., Ltd., USA). Compressive loading was applied at a speed of 1 mm/min until a strain of 20 %. Specimens were kept wet by dropping a saline solution (Otsu normal saline; Otsuka Pharmaceutical Co., Ltd., Japan). The test was conducted for three specimens at each concentration and the Young’s modulus $E$ was calculated from 5 % to 15 % strain.

### 4. Results

Figure 4(a) shows typical displacement images in the $x$-$y$ plane for $x$, $y$, and $z$ directions at frequency of 250 Hz and amplitude of 0.5 mm. Figure 4(b) shows the viscoelastic modulus ($G'$ and $G''$) distributions in the $x$-$y$ plane for 0.6 and
2.0 wt% agarose gels at excitation amplitude of 0.5 mm. The value of $G'$ and $G''$ of each pixel in the images was calculated from Eqs. (4) and (5). The average and standard deviation of $G'$ and $G''$ in the $x$-$y$ plane were shown below the distribution images. The wavelength clearly changed with the agarose gel stiffness. The distribution images of $G'$ were homogeneous in all measurements.

Figure 5 shows the relationship between the $G'$ or $G''$ and excitation amplitudes under the excitation frequency of 250 Hz. The values of $G'$ and $G''$ indicate the average and standard deviation of three specimens. The values of $G'$ increased with the amplitude. The slopes of the relationship between $G'$ and excitation amplitude varied in 7.1 kPa/mm (0.6 wt%), 6.5 kPa/mm (0.8 wt%), 22.0 kPa/mm (1.2 wt%), 23.3 kPa/mm (1.6 wt%), and 29.2 kPa/mm (2.0 wt%). The slope for the 2.0 wt% gel increased by 4.1 times compared with that of the 0.6 wt% gel. The tendency was clearly different between 0.8 wt% or less and 1.2 wt% or more gels.

Figure 6 shows the relationship between the $G'$ or $G''$ and excitation frequency at the excitation amplitude of 0.5 mm. The values of $G'$ and $G''$ indicate the average and standard deviation of three specimens. The values of $G'$ increased with the frequency. The slopes of the relationship between $G'$ and frequency varied in 0.028 kPa/Hz (0.6 wt%), 0.013 kPa/Hz (0.8 wt%), 0.063 kPa/Hz (1.2 wt%), 0.063 kPa/Hz (1.6 wt%), and 0.087 kPa/Hz (2.0 wt%). The tendency was also different between 0.8 wt% or less and 1.2 wt% or more gels. The slope of for the 2.0 wt% gel
increased by 3.1 times compared with that of the 0.6 wt% gel. The $G''$ was a quite small (0.1 ± 0.2 kPa) in all the experiments.

The $G'$ is an elastic component of the complex shear modulus. The $E$ for nine specimens was obtained from the
stress–strain curve of the static compression test: $E_{0.6\text{wt\%}} = 10.0 \pm 1.1 \text{kPa}$, $E_{0.8\text{wt\%}} = 17.9 \pm 2.4 \text{kPa}$, $E_{1.2\text{wt\%}} = 38.6 \pm 7.1 \text{kPa}$, $E_{1.6\text{wt\%}} = 54.8 \pm 8.7 \text{kPa}$, and $E_{2.0\text{wt\%}} = 89.1 \pm 16.8 \text{kPa}$. The relationship between $G'$ (kPa) and $E$ (kPa) was analyzed as an experimental equation with the excitation frequency $f$(Hz) and excitation amplitude $a$ (mm) by multiple linear regression ($R^2 = 0.64$, $p < 0.001$):

$$G' = 0.03E + 0.04f + 13.8a - 4.28.$$  

Figure 7 shows the displacement images of each direction in the $x$-$y$ plane and the viscoelastic modulus images of the type B–D specimens in Fig. 3 at excitation amplitude of 0.5 mm and excitation frequency of 250 Hz. MRI magnitude images indicated the different gel regions. The value of $G'$ in the hard and soft parts increased with the excitation frequency and amplitude in type B–D specimens. Figure 8(a) shows the soft and hard parts in MRI and $G'$ images of type B–D specimens. The dash lines indicated in the $G'$ image were traced using the interface of the gels appeared in the MRI images. Figure 8(b) shows the average of $G'$ in the soft (0.6 wt%) and hard (2.0 wt%) parts of three specimens in type B–D to compare with type A at the same excitation conditions. The difference of $G'$ in the 0.6 wt% gels between type A and type B was up to 0.9 kPa. On the other hand, the difference of $G'$ in the 2.0 wt% gels was larger than the 0.6 wt% gels. The difference of $G'$ between type A and type B was 3.6 kPa (minimum) and between type A and type D was 7.8 kPa (maximum).

5. Discussion

In this study, the viscoelastic modulus of agarose gels with the concentrations of 0.6–2.0 wt% was measured by the MRE system under the excitation frequency of 50–250 Hz and the amplitude of 0.1–0.5 mm. Furthermore, three kinds of the double-layer specimen were examined as an application of the MRE system.

As shown in Fig. 4, the wave patterns were observed in whole the $x$-$y$ plane under the high frequency and large amplitude. The wave pattern was also observed in the other conditions, although it was hard to observe clear patterns in the soft gels under the high frequency and small amplitude. The MRE system obtained the distribution of the displacement at the measured plane in the agarose gels used in the study. It may also suggest the low viscosity of the specimen. Indeed, the values of $G''$ were much smaller than $G'$ under all experimental conditions.

The present study examined gels of five kind of stiffness and investigated the relationship between the values and excitation conditions. Young’s modulus of the gels with each concentration was measured by the static compression tests and the difference of the stiffness with the concentrations was confirmed. The difference of $G'$ between hard (1.2, 1.6, and 2.0 wt%) and soft (0.6 and 0.8 wt%) gels was obtained significantly under the high frequency and large amplitude, as shown in Figs 4, 5, and 6. The average of $G'$ in 1.2 wt% concentration under the excitation frequency of 250 Hz and amplitude of 0.5 mm was $18.4 \pm 2.3 \text{kPa}$ (n=3), and the standard deviation of $G'$ at the $x$-$y$ plane in a specific specimen was $3.1 \pm 0.3 \text{kPa}$ (n=3). On the other hand, the average and standard deviation of $G'$ in a specimen under the same experimental conditions was $17.4 \pm 13.5 \text{kPa}$ in previous measurements (Tadano, et al., 2012). The variation of $G'$ in the $x$-$y$ plane was decreased by using the calculation method proposed in previous study (Jiang and Nakamura, 2011). The standard deviation also might depend on the density distribution in each specimen generated in the specimen preparation. Furthermore, as expressed in Eq. (6), it was confirmed that $G'$ increased with the frequency and amplitude. Riek et al. (2011) reported that $G'$ of a gelatin gel measured by MRE increased with the frequency between 100 and 800 Hz. Feng et al. (2013) also measured the $G'$ and $G''$ of ferret brains in vivo by MRE in the excitation frequency of 400–800 Hz and that the complex shear modulus increased with the frequency. The viscoelastic modulus of agarose gels was measured using the MRE system under the excitation conditions.

Furthermore, the study examined three kinds of double-layer specimens as an application of the MRE system. As shown in Fig. 7, the difference of wave pattern between the hard and soft parts was observed. The wavelength of the pattern within the soft part in the displacement images was shorter than that in the hard part, corresponding to the type A specimen. It would appear that the interface of the gels was observed at almost the same position in the $G'$ images according to Fig. 8(a); however, to validate the distribution, a detection method of the hard region in the $G'$ images and the quantitative comparison with MRI images are required in further studies. In addition, the values of $G'$ in the soft parts of the type B–D specimens corresponded to the value of the type A, but the values of $G'$ in the hard parts were varied in the three types and different from the type A. Perreard et al. (2010) discussed the relationship between an
excitation position and the shear modulus of composite soft matter measured by MRE. To investigate the effects of excitation position or contact condition between the specimen and vibration transmitter on the displacement patterns will contribute to further improvement of the system.

The present study applied the MRE instruments using 0.3-T micro-MRI that was proposed in the previous studies to the measurements of viscoelastic modulus of agarose gels as an example of soft matters matter. The measurements were conducted within the excitation frequency of 50–250 Hz and the amplitude of 0.1–0.5 mm depending on the performance of the bar-type transmitter, the vibration generator, and MRI instrument used here. Although the wave pattern was observed and the difference of $G'$ with the stiffness of the gels was confirmed under those conditions, other concentrations and other materials should be investigated in further studies. Furthermore, the specimen size was set as 90 × 70 × 50 mm in the study. The effects of specimen size on the values and errors of viscoelastic modulus did not investigated here. In addition, the study focused on the x-y plane in the specimens.

In summary, the present study measured the viscoelastic modulus of agarose gels with five kinds of stiffness by the MRE system. The value of $G'$ in the agarose gels and dependence of frequency and amplitude were demonstrated. Furthermore, double-layer specimens composed of different stiffness gels were examined as an application of the MRE system.

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