Characterizing the viscoelastic properties of a tissue mimicking phantom for ultrasound elasticity imaging studies

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Abstract. With the increased use of ultrasound (US) imaging in biomedical fields, the ability to provide phantoms that are capable of mimicking desired properties of soft tissues is important. Polyvinyl alcohol (PVA) is a polymer of great interest because of its relatively simple chemical structure, ease of processing, and satisfactory acoustic and durability properties. PVA may be useful as a phantom material in the study of ultrasound elasticity imaging (UEI) for liver fibrosis. A PVA-based US phantom was constructed through a freeze–thaw process, and measurements of mechanical properties of PVA were carried out with a rotational rheometer. The storage modulus and the loss modulus of PVA were obtained, and the elasticity and viscosity of PVA phantom were estimated by using Voigt viscoelastic model. The obtained viscoelastic results were compared with those of liver at fibrosis stage. Our study suggests that it is possible to construct a PVA based phantom that mimics fibrous liver for the purpose of UEI imaging studies.

1. Introduction

The use of phantoms in ultrasound (US) imaging research is very common. US phantoms emulate acoustic properties of biological tissue and are useful for the development and characterization of US imaging systems or algorithms. Acoustic parameters such as speed of sound, acoustic attenuation coefficient, and acoustic backscatter coefficient are considered as important parameters when constructing a tissue-mimicking phantom for US imaging. Gelatin, polyvinyl alcohol (PVA), agarose, and polyacrylamide are the most commonly used US phantom materials [1]. With the increased use of ultrasound elasticity imaging (UEI) in preclinical and clinical practice in recent years, US phantoms with desirable mechanical properties are needed for the evaluation of the accuracy, repeatability and reliability of UEI algorithms.

PVA gels are excellent tissue substitutes [2] that have good acoustic [1] and durability properties [3]. They are non-toxic, of reasonable cost, and are relative ease of fabrication. Besides, they exhibit elastic and bioadhesive characteristics and have been shown to have potential in various biomedical applications such as articular cartilage, artificial skin, artificial pancreas, etc. In addition, there is an increasing interest in the use of PVA as a tissue phantom for the development of biomodeling [4] and medical imaging, e.g., in photoacoustic mammography [5] and in magnetic resonance imaging [6].

In this paper, PVA was used to make a viscoelastic phantom via freezing and thawing techniques and the rheological properties were measured. The Voigt viscoelastic model was applied to the rheometer test data. The elasticity and viscosity of the PVA phantom were estimated and compared with the viscoelasticity of the liver at fibrous stage. The implications were discussed afterwards.
2. Materials and methods

2.1. PVA phantom preparation
When preparing the US phantom, PVA with a degree of hydrolysis of more than 99% was used, and the average molecular weight was 85,000 to 140000 (Sigma-Aldrich Inc.). 20 g of PVA powder was poured into 200 ml of ultrapure deionized water and heated. The mixture was uniformly stirred, and alumina powder (Fusion Inc.) was added slowly to increase internal scattering of the US phantom. The alumina powder is 3.6 g in total, and contains equal amount of three different sized powders (1-2 Micron, -44/+10 Micron, Sub-micron). A high-precision electronic balance was used for accurate measurements. The solution was stirred continuously, covered to minimize vapor loss, and heated to 100°C within 20 minutes. After observing the complete dissolution of the powder, it was transferred to a 40°C water bath incubator and pre-cooled for an hour to remove the entrapped air. The solution was then poured into a mold, sealed, and placed in a -20°C refrigerator for 12 h. Afterwards, the mixture was taken out and dissolved at room temperature to yield homogeneous PVA gels. The thawed colloid was carefully cut into uniform sheets of 1 mm thickness for further tests.

2.2. Rheology experiments
Rheology experiments were conducted to measure the dynamic mechanical behaviors of the PVA phantom samples. At a given frequency, a sinusoidal shear strain \( \varepsilon(t) = \varepsilon_0 e^{i\omega t} \) is imposed on a sample to be tested, resulting in a sinusoidal shear stress \( \sigma(t) = \sigma_0 e^{i(\omega t + \delta)} \) [7]. The ratio of \( \sigma(t) \) to \( \varepsilon(t) \) is expressed by complex shear modulus \( G^*(\omega) \):

\[
G^*(\omega) = \frac{\sigma_0 e^{i(\omega t + \delta)}}{\varepsilon_0} = \frac{\sigma_0}{\varepsilon_0} (\cos \delta + i \sin \delta) = G'(\omega) + i G''(\omega),
\]

where \( \sigma_0 \) is shear stress amplitude, \( \varepsilon_0 \) is shear strain amplitude, \( \delta \) is a phase-shifted angle, \( \omega \) is angular frequency, \( G'(\omega) \) is storage modulus, and \( G''(\omega) \) is loss modulus. \( G^*(\omega) \) is related to the viscosity and elasticity by various rheological models. This paper chooses the Voigt model to describe the complex shear modulus \( G^*(\omega) \), and PVA is considered as a linear viscoelastic material. The Voigt model is represented by a dashpot and a spring connected in parallel. The complex modulus of the Voigt model can be expressed as [8]:

\[
G^*(\omega) = E + i \omega \eta,
\]

where \( E \) is a modulus of elasticity, and \( \eta \) is viscosity. In this study, we used a rheometer to measure the storage modulus and loss modulus of PVA phantom samples and analyzed the corresponding viscoelastic parameters.

The rheological properties of two PVA film samples were measured using a ViscotesterIQ rotational rheometer (HAAKE Inc.). First, the rotor parameters of the instrument were calibrated using viscosity standard fluids. After the calibration, the testing error was within 0.5%, and then the viscoelasticity tests of the PVA samples were performed. Each sample was tested for both oscillation amplitude scans and oscillation frequency scans. In each scan mode, each sample was tested for three times. The settings for the scans are as follows:

(1) Oscillation amplitude scan Test spacing: 1.00 mm; Test temperature: 20°C; Time for balance: 300 s after loading; Frequency: 1 Hz; Deformation control mode; Strain sweep range: 0.1000%~20%.

(2) Oscillation frequency scan Test spacing: 1.00 mm; Test temperature: 20°C; Time for balance: 300 s after loading; Shear rate: 1.000%; Deformation control mode; Frequency sweep range: 1~20 Hz.

The stress-strain relations were then plotted and fitted, and the viscoelastic parameters were analyzed and compared with published literature.
3. Results and discussion

Figure 1 shows the relationship between stress and strain in the three oscillation amplitude scan tests of sample 1 and sample 2, respectively. The ratio of stress to strain in Fig. 1 is in the range of 1.01–1.69 kPa, with standard errors all less than 0.13 kPa. The $R^2$ values for linear fits are all above 0.98. Different point spacings were used in scan tests of sample 2 to check if they have an effect on the results. From Fig. 1, the results based on the same sample are generally consistent. The choice of point spacing has no significant effect on the scan results. Oscillation amplitude scan tests indicate that sample 2 is slightly stiffer than sample 1.

![Figure 1. The stress-strain relations of the PVA film samples. (a) Sample 1 (b) Sample 2.](image1.png)

Figure 1. The stress-strain relations of the PVA film samples. (a) Sample 1 (b) Sample 2.

Figure 2 shows the relationship between the storage modulus, loss modulus, composite viscous modulus and frequency for sample 1 and sample 2. The measured storage moduli and loss moduli are frequency dependent, but can be used to estimate elasticity and viscosity of the samples through Voigt model. Elasticity was calculated by taking the average of the storage modulus, and viscosity was calculated by using $G'' = \omega \eta$ and then taking the average. The composite viscous modulus curve is a monotonically decreasing function of frequency for both samples. From Fig. 2, oscillation frequency scan tests also reveal that sample 2 is slightly stiffer than sample 1.

![Figure 2. The relationship between the storage modulus, loss modulus, composite viscous modulus and frequency for each sample. (a) Sample 1 (b) Sample 2](image2.png)

Figure 2. The relationship between the storage modulus, loss modulus, composite viscous modulus and frequency for each sample. (a) Sample 1 (b) Sample 2

Table 1 shows the elastic and viscous results obtained from rheology experiments by using the Voigt model. The results show that the PVA phantom we constructed has an elasticity of $1.34\pm0.28$ kPa, and viscosity of $9.67\pm3.00$ Pa•s. Literature [7] measured the dynamic viscoelastic properties of
rat livers in different fibrosis stages F0 to F4. The elasticity of rat livers obtained from fibrosis stage F2 was 0.911±0.555 kPa, and the viscosity was 4.625±1.296 Pa·s [7]. Literature [9] performed multifrequency MR elastography, and the measured elastic modulus and viscosity of fibrous liver were 2.91±0.84 kPa and 14.4±6.6 Pa·s, respectively. The elasticity and viscosity of the PVA phantom we constructed were basically consistent with the fibrous liver.

The viscoelasticity properties can be customized for a specific liver fibrosis stage by adjusting the concentration of the aqueous PVA solution or by increasing cycles of freezing and thawing. As the number of freezing/thawing cycles is increased, denser structures will be observed within PVA gels, and the phantom will become stronger with larger mechanical integrity. There are many other factors that can impact the mechanical properties, like the molecular weight of the polymer, the temperature and time of freezing and thawing, etc.

Table 1. Viscoelastic results obtained from rheology experiments.

| Sample No. | Test No. | Elasticity (kPa) | Viscosity (Pa·s) |
|------------|---------|-----------------|-----------------|
|            |         | Oscillation amplitude scan | Oscillation frequency scan | Oscillation frequency scan |
| Sample 1   | Test1   | 1.037           | 1.209           | 6.010           |
|            | Test2   | 1.009           | 1.178           | 8.807           |
|            | Test3   | 1.048           | 1.173           | 8.648           |
| Sample 2   | Test1   | 1.241           | 1.841           | 14.986          |
|            | Test2   | 1.687           | 1.642           | 10.709          |
|            | Test3   | 1.395           | 1.568           | 8.880           |
| Mean ± SD  |         | 1.34±0.28 (kPa) | 9.67±3.00 (Pa·s) |

Zener model is expected to be the best model for estimating the moduli with the minimum errors [7, 8]. In this paper, we chose to use Voigt model because the moduli of the Zener model cannot be used to describe degree of liver injury directly [8], and there is no dramatic difference in assessing liver fibrosis using the two models [7]. Strain sweep range in the oscillation amplitude scan is set to be within 20% because the use of UEI is generally within 20% surface-applied strain which corresponds to a small and linear deformation [10, 11].

Our study had some limitations. First, only two samples were studied. More experiments are necessary to validate the reproducibility of our results. Second, the real term of the Voigt equation is constant at all frequencies does not perfectly describe the experimental data. Third, only small deformation and linear viscoelasticity was studied. Last but not least, how alumina powders added as ultrasound scatterers influence the overall mechanical property of the PVA phantom is not quite clear. Despite of these, it is expected that PVA phantom constructed using our method is useful in simulating fibrous liver in vitro for UEI algorithm studies and modifications are possible by simple protocol adjustments for simulating different liver fibrosis stages.

4. Conclusion

We have demonstrated that a PVA based phantom can be constructed to simulate fibrous liver for UEI imaging studies. Aqueous solutions of 10 wt% PVA were utilized to construct the phantom through a freeze–thaw process. Mechanical behaviors of the PVA gel were quantified through rheological experiments. The viscoelastic properties of the PVA phantom were analyzed by using Voigt model. Despite of limited number of samples and data points, reasonable results were obtained which indicate that it is possible to use the prepared phantom in the study of liver fibrosis evaluation with UEI. Careful manipulation of the concentration of PVA and repeated cycles of freezing/thawing may help create a tissue-mimicking phantom with desirable properties that can simulate liver fibrosis at different stages for the continued development of UEI algorithms.
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