Tensile Testing with Vessel for Pressure Dependency Evaluation of Viscoelasticity of Biological Soft Tissue*

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Abstract
The mechanical behaviors of solid-liquid two-phase materials are affected by environmental conditions because of the moist structure of the materials. Therefore, material tests to investigate the solid-liquid interactions in these materials should be conducted by considering various conditions such as multiaxial stress, temperature, and fluid concentration. Here, the deformation of biological soft tissues having high liquid content is also dependent on multiaxial conditions including hydrostatic stress. However, it is difficult to evaluate the condition dependency of tissues because no equipment has been developed to realize low-load sensing under hydrostatic stress conditions. In this study, a pressure vessel for a tensile testing system is developed to evaluate the pressure dependence of biological soft tissues. This vessel can be attached to the tensile testing system that can be used to evaluate the viscoelasticity of tissues. A non-friction sealing system with a film on the vessel wall is adopted to measure the load; this load is verified by carrying out a tensile test with a linear spring under pressure conditions. In particular, the error caused by the opened/closed condition of the vessel is calibrated by the verification results, and the load applied to the specimen is precisely measured by using the tensile system with the pressure vessel. After the calibration, a tensile test for biological soft tissue is carried out under varying pressure conditions. The experimental results indicate that the stress-time curves for each condition exhibit good agreement and the tensile system with the pressure vessel has good applicability to evaluate the pressure dependency of viscoelastic behavior.

Key words: Pressure Vessel, Viscoelastic Deformation, Tensile Properties, Deformation Characteristics, Soft Tissue

1. Introduction
The mechanical behaviors of biological soft tissues in which cells have high liquid content are related to the characteristics of the liquid phase. In particular, there have been numerous reports on the simulation of the compression behavior of solid-liquid two-phase materials[1]–[3]. On the other hand, it is necessary to evaluate the compression and tensile properties of soft tissues on the basis of the dynamics of human motion. Therefore, compression[4],[5] and biaxial tensile tests[6] of soft tissues have been conducted. However, there have been few reports on tensile tests of soft tissues under conditions of pressure[10] although there has been considerable research on metallic materials[7]–[9]. In particular, low-load sensing without the influence of pressure is difficult; however, a technique is required to precisely evaluate the deformation of soft tissues. In this study, a pressure vessel is developed to evaluate the pressure...
dependence of viscoelasticity in biological soft tissues. The developed vessel can be attached to a tensile testing system(11) to observe the viscoelastic deformation of a soft viscoelastomer. In this case, a load cell is positioned outside the vessel and a non-friction sealing system is developed to measure the load applied to specimens in the vessel. A calibration technique for load sensing is also described in this paper. The results of tensile tests carried out under varying pressure conditions for biological soft tissue are discussed to investigate the applicability of the vessel to the evaluation of the pressure dependency of viscoelasticity in soft tissues.

2. Tensile Testing System

2.1. Behavior of Biological Soft Tissue and Evaluation of Pressure Condition

The mechanical behavior of solid-liquid two-phase materials such as biological soft tissues is evaluated by using the principle of effective stress(1). The compression behavior of a viscoelastomer is represented by a model in which a liquid phase is moved in solid phase by differential pressure. On the other hand, the stress response of a viscoelastomer is usually nonlinear, as indicated by the solid line in Fig.1; this figure shows a schematic of the tensile response with a constant strain rate. Additionally, asymptotic stress relaxation is also observed, as indicated by the dashed line in Fig.1. Therefore, it is necessary to evaluate the pressure dependency of materials in order to analyze the process of viscoelastic deformation.

However, the testing system required to observe a stress-time curve such as that shown in Fig.1 is large because the range of the strain rate is wide and a function for realizing an abrupt stop is required to observe asymptotic stress relaxation. Therefore, a pressure vessel that can be attached to various testing parts of the system is developed; this vessel has a limited size.

![Fig. 1 Schematic of stress-time curve of viscoelastomer under tensile load at constant strain rate and stress relaxation.](image)

2.2. System Specifications

A schematic of the tensile testing system is shown in Fig.2. A mechatro-actuator, XY-HRS063-RS204 (NSK Ltd.), is used as the driving device for the system; this device can move from 0.1 to 1200 mm/s. A specimen is connected to the sensing and loading rods by using an instant adhesive. The tensile test can begin at setting speed because the loading rod is connected to the table after the acceleration to the speed. The rod is also disconnected when setting strain is added to the specimen and stress relaxation can be observed by monitoring the stress variation after disconnection. In the tests, the load applied to the specimen is measured through the sensing rod by the load cell, LTS-1KA (KYOWA Elect. Inst. Co., Ltd.). The displacement of the loading rod is measured using a laser displacement sensor, LB-62 (KEYENCE Corp.). The developed pressure vessel is attached to the testing part of the tensile testing system as shown in Fig.3.

3. Pressure Vessel

3.1. Features

A schematic of the developed pressure vessel is shown in Fig.4(a). The vessel is made of acrylic resin and it has a capacity of 0.9 L. The loading and sensing rods penetrate the side
Fig. 2 Schematic of tensile testing system.

Fig. 3 Schematic of entire testing system with pressure vessel.

3.2. Condition Control

The pressure vessel can control experimental conditions such as the temperature, liquid, and pressure. The pressure is controlled by using a pump, T-100K (KYOWA, Ltd.). For pressure control, a weight is settled to maintain a setting pressure on the pump. A pressure gauge, PAB-A-1MP (KYOWA Elect. Inst. Co., Ltd.), is connected on the sidewall of the vessel, and its data is logged on a PC. The valve on the top of the vessel can be used to close/open the vessel to the atmosphere. The temperature is controlled by using a ceramic heater and a thermocouple, TF-C11 (KEYENCE Corp.), and the ON/OFF control circuit of a temperature controller, TF-4-10V (KEYENCE Corp.), is used to control the temperature of the liquid by using the ceramic heater at an alternating voltage of 100 V. The temperature controllability of this system is within the range of 0.4 °C when the target temperature is 30 °C for water.

3.3. Load Sensing

A schematic of the non-friction sealing system for the sensing rod is shown in Fig.5. The closing of the vessel is realized by a flexible resin film. The flexibility is satisfied with the non-friction sensing of the load to the specimen which is inside the vessel. When investigating the deformation behavior of the specimen, it was found that the pressure $P$ applied to the vessel liquid causes film deformation, as shown in Fig.5 (a), and it also causes the load cell to shift. The load cell is fixed at the shifted position, following which the load $L$ applied to the specimen can be measured without the influence of any friction and tension force of the wall of the vessel and they are used to measure the external load applied to specimen. The hole through which the loading rod penetrates is sealed using a rod seal, SPNC-3 (NOK Corp.). The hole through which the sensing rod penetrates, on the other hand, is sealed using a non-friction sealing system because it is necessary to precisely measure the load by eliminating the friction effect\(^{[12]}\)\(^{\text{−}[14]}\). The developed sealing system is made of a resin film and an O-ring, 1BP-3 (NOK Corp.), as shown in Fig.4(b), and the sensing rod is adhered to the O-ring by using an adhesive.
Fig. 4 Schematic of pressure vessel (a) and photograph of the non-friction sealing of the vessel (b).

Fig. 5 Effect of pressure on sealing film for sensing rod.

Fig. 6 shows stress-displacement curves obtained in the tensile tests carried out for calibration. The tests are conducted under fixed pressure conditions, and the differences caused by varying conditions can be observed here. In this result, the results

film $T$, as shown in Fig. 5 (b), because the tension $T$ acts on the sensing rod along a tangential direction.

3.4. Calibration

3.4.1. Procedure  The measurements obtained using the testing system with the pressure vessel are calibrated in order to realize precise evaluation of the materials. Here, the influence on load sensing in the system is inspected by using a linear metallic spring as the specimen because of its linearity and pressure-independency.

In the inspection, the vessel is filled with tap water as the liquid, and the pressure of the water is controlled by the pump. The pressure condition is varied from 0 to 100 kPa, with the result under an atmosphere without water being considered as the reference. Furthermore, the condition of the valve that is on the top of the vessel is inspected to evaluate the influence of the opened/closed states of the vessel. In the tests, the displacement rate is 0.1 mm/s; this is the minimum displacement rate that can be applied using the driving device.

3.4.2. Calibration Effect  Fig.6 shows stress-displacement curves obtained in the tensile tests carried out for calibration. The tests are conducted under fixed pressure conditions, and the differences caused by varying conditions can be observed here. In this result, the results
obtained for conditions of 0 kPa with an open valve and the atmosphere are compared; however, the results of the closed condition differ from these, as shown in Fig. 6(a). A comparison of the results for 0 kPa conditions indicates that the opened/closed valve condition causes differences. This difference can be caused by the insufficient activity of the pump which has the structure friction of cylinder packing. However, it is difficult to reduce the friction because of the effects of the pump structure.

Then, the calibration is carried out by using the difference between the results of 0 kPa conditions, as shown in Fig. 6(b). Here, the original results for pressure conditions ranging from 0 to 100 kPa with a closed valve are calibrated by subtracting the difference between the results of 0 kPa conditions. By using the indicated calibration, the load measurement under a closed vessel condition is modified to evaluate the viscoelastic behavior of soft tissues.

3.5. Measurement of Viscoelasticity
3.5.1. Experimental Condition The viscoelastic behavior of biological soft tissue is measured by using the developed testing system with the pressure vessel. The experimental conditions used for the tensile test to measure the viscoelasticity are listed in Table 1. Chicken *pectoralis* is adopted as a soft tissue specimen because of its easy availability. The specimens are cut out from an individual piece of chicken *pectoralis* by using a surgical knife, and their size is a minimum muscular unit used for the observability of the experiment.

In the measurement of the specimen dimensions, its cross-sectional shape is considered to be elliptical. The measured diameter of the dimensions is almost 3.7 mm. The chuck span of the specimen is considered as the length of the dimensions, and the specified length of the
specimen is almost 20 mm in this report. In the observation of the viscoelastic behavior, the nominal tensile strain rate is 0.1/s and the application of the tensile strain is stopped at a strain of 0.2 in the loading process. The stress relaxation process at a strain of 0.2 is also observed after loading is stopped. These tests are conducted under the four pressure conditions listed in Table 2. Every piece is used to cut out the specimens that are examined under each pressure condition because of the minimization of individual differences. NaCl solutions are used as the liquid in the vessel, and the concentration of NaCl is obtained as 0.9% from medical literatures. The temperature of the liquid in the vessel is maintained at 30°C to avoid tissue denaturation.

<table>
<thead>
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<th>Strain rate, 1/s</th>
<th>Chuck span, mm</th>
<th>Maximum strain</th>
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<td>0.1</td>
<td>20</td>
<td>0.2</td>
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<table>
<thead>
<tr>
<th>Pressure, kPa</th>
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<th>50</th>
<th>100</th>
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<tbody>
<tr>
<td>Liquid</td>
<td>0.9% NaCl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature, °C</td>
<td>30</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

3.5.2. Result and Discussion The experimental results of the tensile tests carried out to observe the viscoelastic behavior of soft tissue are shown in Fig. 7. Each curve shows the mean stress-time curves obtained in the tests, and error bars indicate the standard deviation of the results. As shown in Fig. 1, the processes of tensile response and asymptotic stress relaxation can be observed in each pressure condition. In this result, it is known that the tissues exhibit wide variation in the processes; however, the mean curves are in good agreement. These results indicate the functional capability of a non-friction sealing system to eliminate the influence of pressure in the vessel, and they validate the calibration. The pressure independency of the viscous behavior of the tissue can be caused by insufficient mobility or the free transition of the liquid phase in the tissue. Therefore, this technique is useful for investigating the biological nature of soft tissues.

![Fig. 7 Mean of stress-time curves of biological soft tissue under pressure conditions.](image)

4. Summary

In this study, a pressure vessel that can be attached to a tensile testing system is developed to evaluate the viscoelastic deformation of biological soft tissue. The results obtained in this study can be summarized as follows:
(1) The pressure vessel is attached to the tensile testing system by adopting a non-friction sealing system with a resin film to measure the external load applied to the specimen.

(2) The tensile testing technique using the vessel is calibrated by a linear spring for precise measurement.

(3) The technique is applied to measure the viscoelastic behavior of biological soft tissue, and its effectiveness is discussed in terms of the pressure dependency of viscoelasticity.

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References


