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# Calibration Method for Elasticity Evaluation of Regenerating Cartilage Based on Ultrasonic Particle Velocity

超音波粒子速度を用いた再生軟骨弾性計測の校正方法の検討

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## 1. Introduction

It is important for regenerative medicine to evaluate the maturity of regenerating tissue. In the maturity evaluation of regenerating cartilage, it is useful to measure the temporal change of elasticity because the maturity of regenerating tissue is closely related to its elasticity. In this study, a quantitative elasticity evaluation for the extracted regenerating cartilage sample, which is based on the laser Doppler measurement of ultrasonic particle velocity and calibration, is experimentally investigated using agar-based phantoms with different elastic moduli and the regenerating cartilage samples extracted from the beagles in animal experiments.

## 2. Method

A measurement system of the regenerating cartilage elasticity is constructed in **Fig. 1**. In this system, an urethane acoustic coupler with a thickness of 10 mm is put on the surface of ultrasound transducer with a center frequency of 1 MHz (GE Sensing & Inspection Tech., 221-340), and an extracted regenerating cartilage sample is put on the acoustic coupler. Ultrasound pulses with a wave number of 5 is irradiated to the bottom of cartilage sample via acoustic coupler. A laser Doppler vibrometer (LDV) (Graphtec, AT0023 & AT3700, frequency range < 10 MHz) is set up at the position of 30 cm apart from the cartilage sample surface, and measures ultrasonic particle velocity on the surface of cartilage sample. The ultrasonic particle velocity waveforms are recorded by using a digital oscilloscope (LeCroy, WS454VL) at a sampling frequency of 500 MHz. After recording the data, the particle velocity waveform is converted to the particle displacement waveform by temporal integration. The displacement is induced by the acoustic pressure of ultrasound pulses. Assuming the applied acoustic pressure is constant and the effect of attenuation is also constant, the displacement reflects the cartilage sample elasticity.

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Actually, since the displacement varies according to the thickness of cartilage sample, the displacement is normalized by the cartilage sample thickness. This processing corresponds to the strain calculation assuming that the displacement at the boundary between the cartilage sample and the acoustic coupler is zero. In the previous study, the elasticity of regenerating cartilage sample was evaluated by the inverse of strain (IS), which is obtained as the ratio of the sample thickness to the particle displacement[1)]. However, the IS is not quantitative for elasticity evaluation although the IS reflects the elasticity. In the previous study, we also found the linear relationship between the IS and the elastic modulus of cartilage sample, by selecting appropriate frequency and thickness of sample[1)]. This linear relationship implies that the following simple calibration is applicable.

$$E = C \cdot \mathrm{IS} \qquad (1)$$

where $E$ and $C$ are elastic modulus and calibration coefficient, respectively. In this study, the above calibration coefficient is determined experimentally, using agar-based phantoms with different elastic modulus. Moreover, the elasticity of regenerating cartilage samples extracted from the beagles in animal experiments is evaluated by using the calibration coefficient.

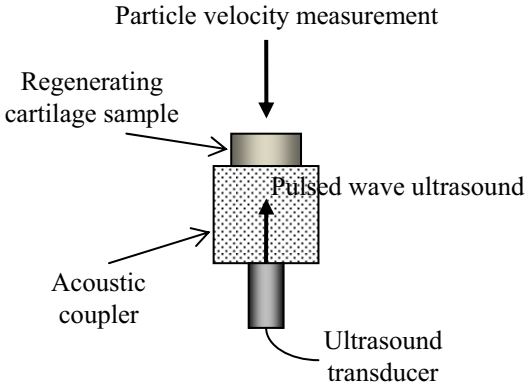


Fig. 1 An elasticity measurement system of the regenerating cartilage sample.

## 3. Determination of Calibration Coefficient

In order to determine the calibration coefficient in this study, three agar-based phantoms with different elastic moduli (0.05, 0.1, 0.2 MPa) and constant size (each side of 10 mm, thickness of 5 mm) were made by changing the weight concentration of agar powder. Since these weight concentrations correlate with elastic moduli of phantoms, these phantoms were used as calibration materials for elasticity evaluation.

Figure 2 shows the comparison between the IS and the elastic moduli measured by the mechanical compression test using an Instron-type universal testing machine (A&D, UTM-10T). The IS coincided well with each measured elastic modulus. In addition, a lenear relationship between the IS and the elastic modulus was observed. Therefore, a calibration coefficient was determined by fitting a linear expression to the measured points in **Fig. 2**.

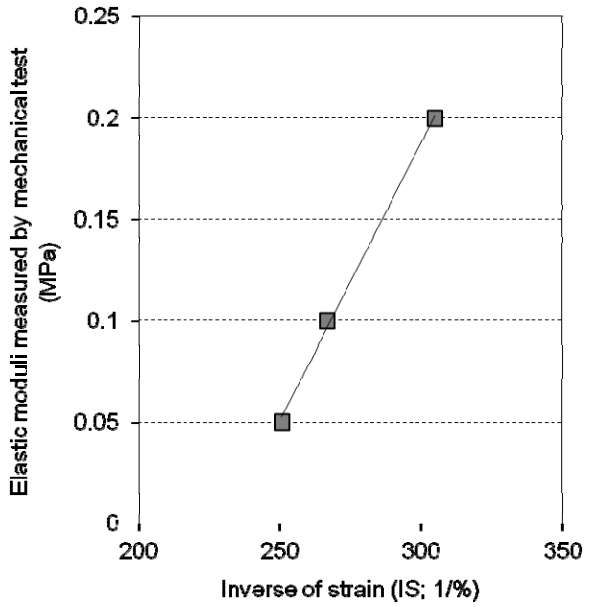


Fig. 2 Determination of calibration coefficient using agar-based phantoms with different elastic moduli.

## 4. Regenerating Cartilage Sample Measurement

In vitro measurements using the regenerating cartilage samples, which were extracted from the beagles in the approved animal experiments, were conducted by using the above-mentioned system. Autologous auricular cartilage cells of the beagle were transfused into the scaffold (PLLA) and cultured for a certain period. The scaffold with the cultured cells were transplanted subcutaneously in the same beagle and extracted after 2 months. The extracted cartilage sample (each side of 5 mm, thickness of 1 mm) was placed on the acoustic coupler, and the elasticity was evaluated as well as the previous phantom measurements.

Figure 3 shows the evaluation result of regeneration cartilage sample elasticity according to the culture periods (1, 2, 3 weeks (wk)). As a reference, control data was also acquired using the only scaffold without any cells. In addition, these results are calibrated by using the calibration coefficient obtained in the previous phantom experiments. Relatively-high correlation between the calibrated elastic moduli and the culture periods of regenerating cartilage samples was observed.

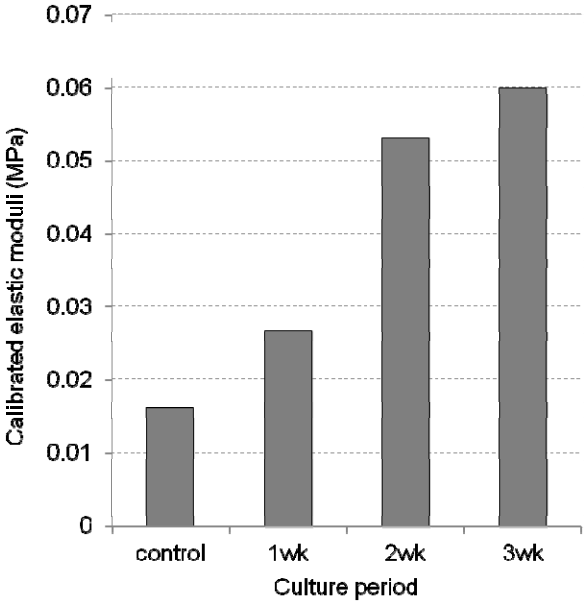


Fig. 3 Calibration results for regenerating cartilage samples.

## 5. Conclusions

The calibration method for quantitative elasticity evaluation of regenerating cartilage sample was investigated using agar-based phantoms and regenerating cartilage samples. In future work, the accuracy of calibration method must be improved through measurements for a number of calibration materials. Moreover, strategies for in vivo measurement of regenerating cartilage must be investigated.

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**References**

1. N. Nitta, M. Misawa, K. Homma and T. Shiina: Jpn. J. Appl. Phys. **51** (2012) 07GF15.