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Elasticity Evaluation of Regenerating Cartilage Sample Based on Laser Doppler Measurement of Ultrasonic Particle Velocity

超音波粒子速度のレーザードップラー計測に基づく再生軟骨片の弾性評価

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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, an elasticity evaluation for the extracted regenerating cartilage sample, which is based on the laser Doppler measurement of ultrasonic particle velocity, is experimentally investigated using the agar-based phantom with different elastic modulus 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. Pulsed ultrasound 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 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 pulsed wave ultrasound. 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. Consequently, the elasticity of regenerating cartilage sample is evaluated by the inverse of the above-mentioned strain, which is obtained as the ratio of the particle displacement to the sample thickness.

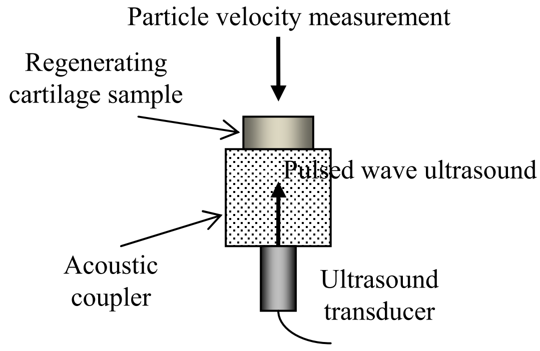

Fig. 1 A measurement system of the regenerating cartilage elasticity.

3. Phantom Measurement

In order to investigate the feasibility of this method to distinguish the elasticity of sample, three phantoms with different elasticity (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 the weight concentration of agar powder correlates with its elasticity, three phantoms simulate the temporal elasticity change according to the maturity of regenerating cartilage sample.

Figure 2 shows examples of particle velocity waveform measured by the LDV. As the elasticity of agar-based phantom increases, slight decrease of waveform amplitude was observed. Figure 3 shows the particle displacement on the surface of each phantom. Here, the horizontal axis indicates the number of measurement and the elastic modulus variation. Since the thickness of each phantom was constant, the displacement decreased according to the increase of elasticity. Figure 4 shows the comparison between the inverse of strain described in Method and true elastic modulus measured by the mechanical compression test. The inverse of strain coincided well with the elastic modulus measured by the mechanical test. That is, this method is capable of evaluating sample elasticity.

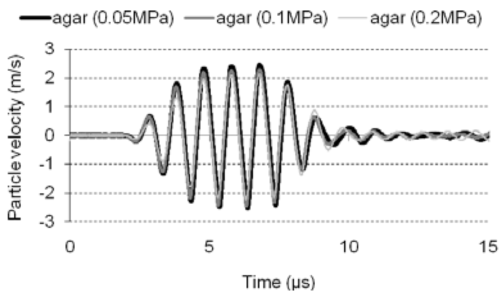

Fig.2 Examples of particle velocity waveform on the surface of agar-based phantom.

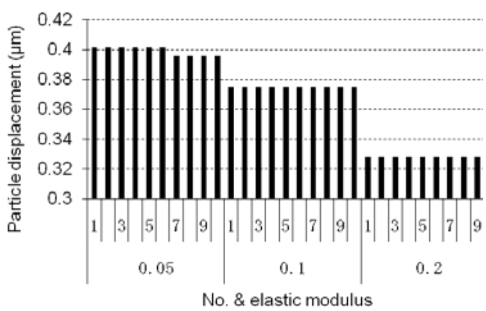

Fig.3 Particle displacement values on the surface of agar-based phantom.

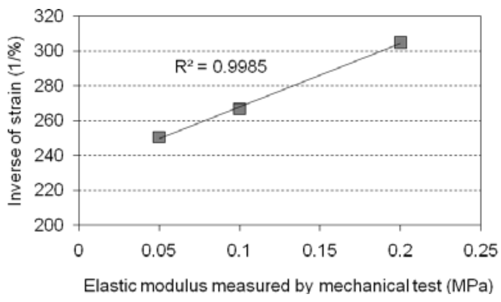

Fig.4 Comparison between the inverse of strain and true elastic modulus measured by the mechanical compression test.

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 5 shows the evaluation result of regeneration cartilage sample elasticity according to the culture period (1, 2, 3 weeks (wk)). Here, ‘control’ means the cartilage regenerated using the only scaffold without any cells. Relatively-high correlation between the inverse of strain and culture period was observed. Also, the inverse of strain coincided well with the elastic modulus measured by the mechanical test (R^2 = 0.9). Therefore, this result in **Fig. 5** implies that the longer culture period regenerates the stiffer cartilage tissue.

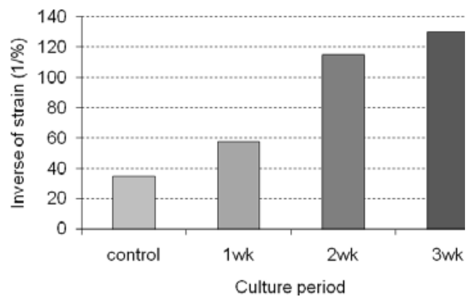

Fig.5 Evaluation result of regenerating cartilage sample elasticity according to the culture period.

5. Conclusions

The feasibility of this method was suggested through the phantom and regenerating cartilage sample measurements. In future work, the effectiveness of proposed method must be verified through measurements for a number of regenerating cartilage samples. Moreover, strategies for in vivo measurement of regenerating cartilage must be investigated.

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