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Application News

Micro Strength Evaluation Testing Machine   Micro Autograph   MST

Evaluation of the Physical Properties of Biological Tissues

Daisuke Hiramaru and Yuki Nishikawa

User Benefits

- Physical properties of moist and flexible biological tissues and biomaterials can be measured.
- The elastic modulus can be evaluated and the hardness can be compared regardless of the size and shape of the specimen.

■ Introduction

Various research institutes and companies are conducting intensive research into therapeutic applications of regenerative medicine. In recent years, various organoids that reproduce properties close to those of living organs have attracted attention, and they are expected to provide innovative treatments for various organ failures that are difficult to treat in modern medicine. Those organoids that reproduce the organ function must be cultured in three dimensions, and scaffold materials for cell engraftment are considered to be one of the important elements for proper tissue culture.

The hardness of scaffold materials used in cell culture is an important element in culture, and there are cases where cell characteristics change according to the hardness of scaffold. The elastic modulus of the specimen is correlated with the hardness. The higher the elastic modulus, the harder the specimen, and the lower the elastic modulus, the softer it is. This article introduces an example of quantitative evaluation of the hardness of scaffold materials and chicken liver by elastic modulus using micro autograph MST.

■ Measurement Specimens

Mighty (CSM-50) and Honeycomb (CSH-96) (Both are collagen sponge products in the AteloCell® series of KOKEN Co., Ltd.), which are scaffold materials for cell culture, and chicken liver, which is a living tissue, were used as measurement specimens. The specimens were immersed in PBS (phosphate buffer saline) from the previous day until just before the measurement to avoid a change in condition due to dryness, and were kept in the same wet condition as when they were cultured.

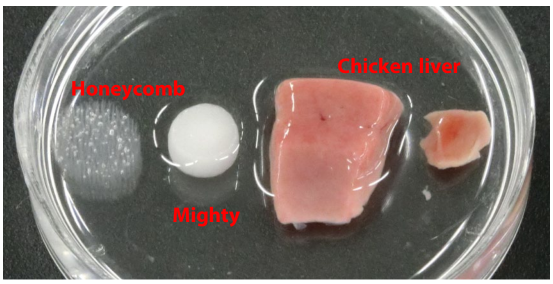

Fig. 1 Scaffolding Material and Chicken Liver

■ Measurement Method

The measurement conditions of this test are shown in Table 1. A push rod with a hemispherical tip was selected for the physical property evaluation described later.

Table 1 Measurement Conditions

Testing Machine	: Micro autograph MST
Load Cell	: 5 N
Test Jig	: Boil-in-bag piercing rod (tip R 0.5 mm)
Test Speed	: 1.0 mm/min

Fig. 2 shows the measurement. The measurement specimen which had been immersed in PBS until just before the test was set on the stage and a piercing test was performed. Using a stereo microscope (optional), it is possible to observe the deformation during piercing in real time.

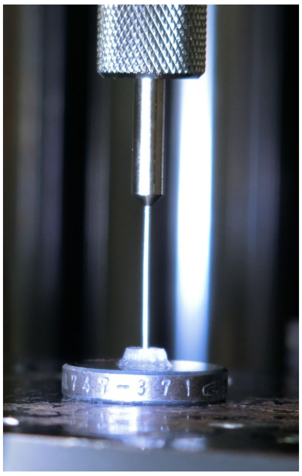

Fig. 2 Photo of Piercing Test

■ Physical Property Evaluation Independent of Specimen Size and Shape

Biological and cultured tissues are often amorphous and may not form a suitable specimen shape. In order to evaluate the hardness of such specimens, a physical property evaluation method that does not depend on the size of the specimen was adopted.

As shown in Fig. 3, Hertz's contact formula[1)] was applied to this test as a contact problem between an infinite plane elastic body and a hemispherical push rod. The relationship between the force and the penetration depth is expressed by Equation (1), and the elastic modulus can be obtained regardless of the size and shape of the specimen.

$$P = \frac{4ER^{1/2}}{3(1-\upsilon^2)}h^{3/2} \Leftrightarrow E = \frac{3(1-\upsilon^2)}{4R^{1/2}h^{3/2}}P \quad \text{... Equation (1)}$$

P : Force received by the push rod, E : Elastic modulus
υ : Poisson's ratio, R : Push rod radius
h : Penetration depth

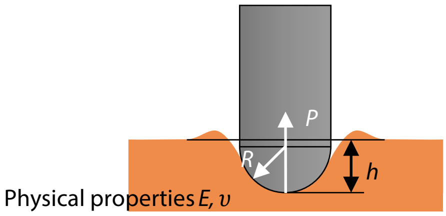

Fig. 3 Model Image of the Test

■ Measurement Results

The results for the three kinds of specimens are shown in Figs. 4 and 5. Each specimen showed a different slope, and the difference in hardness of each specimen is clear.

For the chicken liver, large and small specimens were measured and similar results were obtained as shown in Fig. 1, indicating the possibility that this test method can eliminate the effect of size.

When the force or penetration depth is near 0, the elastic modulus becomes unstable due to the influence of vibration and wind. On the other hand, when the penetration depth is 0.3 mm or more, the force varies especially in Honeycomb and chicken liver. This may be due to the diverse and complex internal structure of the specimen.

Based on the above, the elastic modulus was calculated from equation (1) using the penetration depth and test force in the range of 0.1 mm to 0.3 mm. Poisson's ratio was set to 0. Table 2 shows the calculated results of the elastic modulus for each specimen. The elastic moduli are 635 kPa for Mighty, 12.0 kPa for Honeycomb, and 1.41 kPa for chicken liver. It has been reported in a preceding study that human articular cartilage is 200 – 800 kPa[2,3] and human liver is 0.5 – 7 kPa[4], and it can be said that the measurement method is appropriate for the physical property evaluation of various biological tissues.

Table 2 Average Elastic Modulus of Each Specimen

Specimen	Mighty	Honeycomb	Chicken liver
Elastic Modulus [kPa]	635	12.0	1.41
Elastic Modulus ratio	449	8.52	1

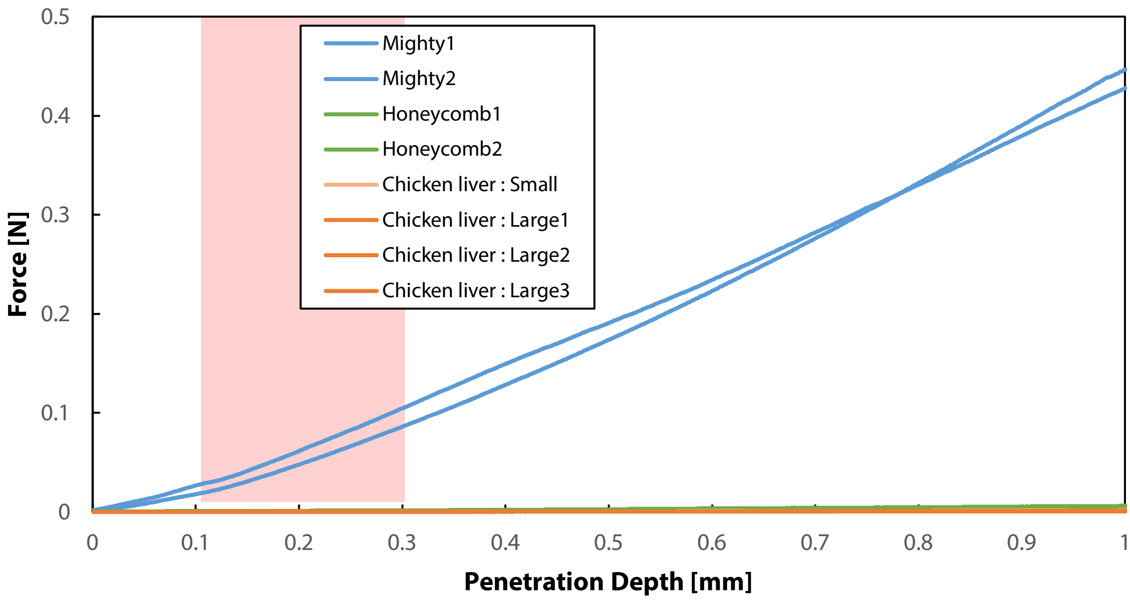

Fig. 4 Force-Penetration Depth Diagram for Piercing Test

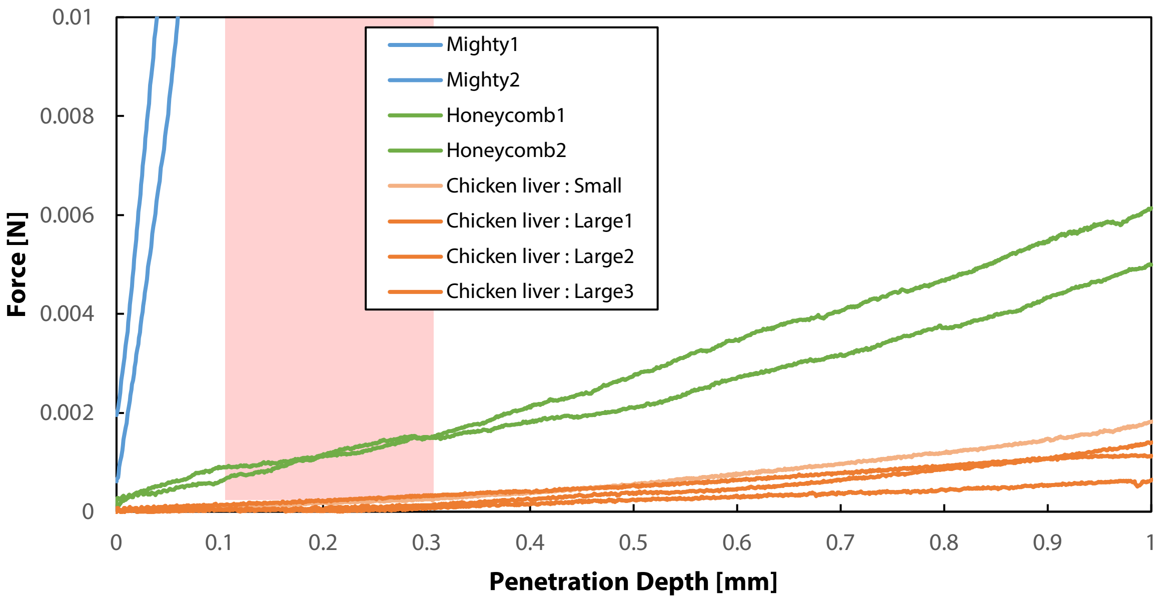

Fig. 5 Force-Penetration Depth Diagram for Piercing Test (Enlarged View of Low Force Region)

■ Consideration of Adaptive Size Limit

Equation (1), which shows the relationship between the force and the penetration depth using the Hertzian contact equation, assumes the specimen shape to be an infinite plane, so it is expected that it will not hold for extremely small or thin specimens. Therefore, the size limit for which equation (1) holds was investigated using FEM analysis.

Under the condition that the penetration depth is 0.2 mm, which is the median value of the calculated range of the elastic modulus, a cylindrical specimen is pierced as shown in Fig. 6. Here, using the specimen radius *r* and specimen thickness *t* as variables, the force *P* for piercing was calculated by simulation. By comparing the results with the force P_∞ obtained for a specimen with a size sufficient to be considered infinite (r = 100 mm, t = 100 mm), it is possible to determine how much the apparent elastic modulus changes with the specimen size. Table 3 shows the conditions used in the simulation.

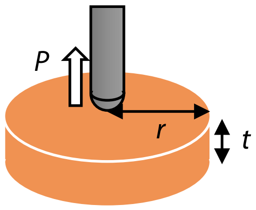

Fig. 6 Model Image for FEM Simulation

Table 3 Physical Property Conditions Used in the Simulation

Material Property	Assuming to be an elastic body
Push Rod Radius *R* [mm]	0.5
Penetration Depth [mm]	0.2
Elastic Modulus *E* [kPa]	40 *Organ model estimates
Poisson's Ratio υ	0

The simulation results are shown in Fig. 7. For the thickness *t*, it was found that the apparent hardness was evaluated as 5 % harder than the actual hardness when the thickness was less than approximately 6 mm, and 10 % harder when the thickness was less than approximately 3 mm. It was also found that the threshold value at which the material was evaluated to be 1 % softer had a linear relationship with the radius *r* and the thickness *t*. This may be due to the aspect ratio. From these results, it is clear that equation (2) must be satisfied as the criterion for the practical applicability of equation (1).

$$\left.\begin{array}{c} t < 2r - 2.8 \\ t > 6 \end{array}\right\} \quad \text{… Equation (2)}$$

In this study, in order to evaluate the effect of the specimen elastic modulus, a simulation was also conducted under the condition of elastic modulus 15 MPa, and the same result was obtained. For this reason, Equation (2) can be considered as a conditional equation applicable to all specimens in this test.

■ Conclusion

The piercing test using the MST can easily and quantitatively evaluate the physical properties of moist and flexible biological tissues and biomaterials. Furthermore, by using the Hertzian contact method, it is possible to compare the elastic modulus (hardness) regardless of the size or shape of the specimen. In addition, the validity of this test was confirmed by clarifying the applicable range of the Hertz contact equation used in this test through simulation.

References

1) Xinyao Zhu, E. Siamantouras, K. K. Liu, X. Liu, J Mech Bihav Biomed Mater, 56, 77-86
2) K. A. Athanasiou, M. P. Rosenwasser, J. A. Buckwalter, T. I. Malinin and V. C. Mow, J Orthop Res, 9, 330-40
3) F. Boschetti, G. Pennati, F. Gervaso, G. M. Peretti and G. Dubini, Biorheology, 41, 159-66
4) W.-C. Yeh, P.-C. Li, Y.-M. Jeng, H.-C. Hsu, P.-L. Kuo, M.-L. Li, P.-M. Yang and P. H. Lee, Ultrasound in Med. & Bio., 28, 467-474

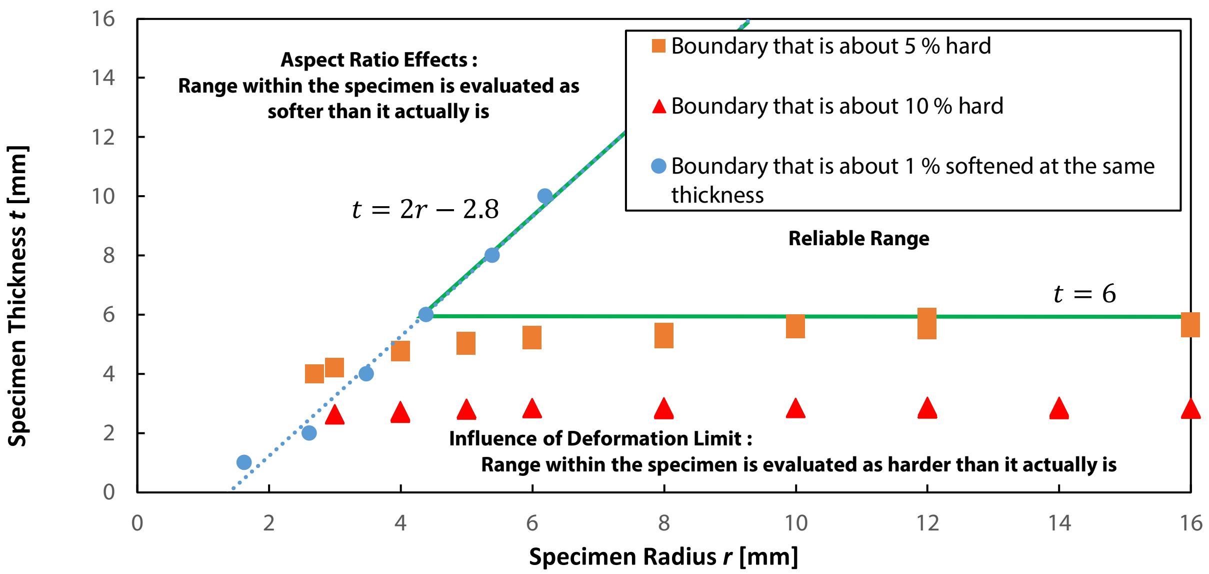

Fig. 7 Evaluation of the Effect of Thickness and Radius on Force

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