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Application News

No. i285

Micro Compression Testing Machine

Compression Tests for Cell Aggregates

In recent years, various research institutes and companies have been actively researching the therapeutic applications of regenerative medicine. In regenerative medicine, injuries and illnesses are treated by culturing the desired cells and tissues in vitro and then transplanting them . There are high hopes for the potential of this approach to cure illnesses that are currently hard to treat. In 2014, retinal pigment epithelial cells were transplanted in the world's first clinical study using human iPS cells. Since then, clinical studies have been conducted using cultured neurons, cultured chondrocytes, and cultured myocardial cell sheets, indicating that regenerative medicine is moving beyond the research stage to clinical applications. However, realizing such applications involves a number of issues. An important one is to establish quality control techniques for the cultured cells and tissues used for transplantation. At present, we rely on experienced researchers to make acceptability judgments. In addition, to achieve widespread use, quality control methods are needed to evaluate the characteristics of cultured cells and tissues quantitatively.

As an example, this article introduces the measurement of deformation strength[*1] as an equivalent to the hardness of cell aggregates, a model for cultured tissues. The Shimadzu MCT™ micro compression testing machine is suited to compression tests on microscopic samples and can handle even flexible cell aggregate samples. Generally, cells and tissues are fragile, and quantitative mechanical evaluations using testing machines are difficult. However, the MCT enables quantitative evaluations of deformation strength thanks to its high accuracy displacement detection and force measurement. We expect that quantitative evaluation using high accuracy testing machines will contribute to the spread of regenerative medicine.

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[*1]: Deformation strength
The strength is calculated from formula (1) on page 2 of this article. It is defined in JIS Z 8844 (Test method of fracture and deformation strength of a fine particle)

■ Samples

In this test, cell aggregates were created from three types of cell, HEK293 and iPS cells A and B (human iPS cell strains). HEK293 is derived from human embryonic kidneys and is a general purpose cell line. iPS cells A and B are iPS cell strains established using the same methods with cells derived from different humans.

A low adhesion U-bottom 96-well plate was used for forming cell aggregates from cells of the three types. Table 1 shows the reagents used. The details of the formation method are as per Table 2. The cell aggregates were deactivated by immobilization before testing.

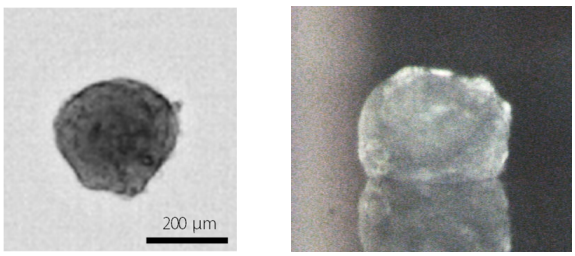
Fig. 1 shows the formed cell aggregates. It is evident that cell aggregates with a suitable spherical shape were obtained.

Table 1 Reagents Used

Incubator	:	Low adhesion U-bottom 96-well plate
HEK293	:	Culture media: D-MEM, FBS
iPS Cells	:	Culture media: AK02N, ROCK inhibitor: Y-27632
Reagent Used for Immobilization	:	4 % paraformaldehyde

Table 2 Cell Aggregate Formation Procedure

1	Disseminate cells in a 96-well plate (1×10^3 cells/well).
2	Culture for 4 days.
3	Immobilize using paraformaldehyde.
4	Store inside PBS.

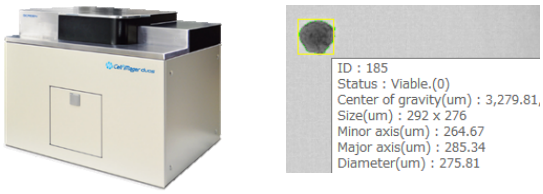


a. Top (In Solution) b. Side (In Atmosphere)

Fig. 1 Cell Aggregate (HEK293)

■ Cell Aggregate Particle Size Measurements

The Cell³iMager duos (SCREEN) was used to evaluate the shape of the cultured cell aggregates. This instrument can simultaneously acquire the shape information of cultured cell aggregates in a 96-well plate. The analysis of about 200 wells (two 96-well plates) were completed within 4 minutes, making it very convenient to acquire the cell aggregate size information required for this study. Table 3 shows the average sizes obtained for three cell aggregate types, HEK293 and iPS cells A and B. Particle sizes measured with this method were used to calculate the deformation strength.



a. Instrument **b. Example of the Analysis Results of the Images**

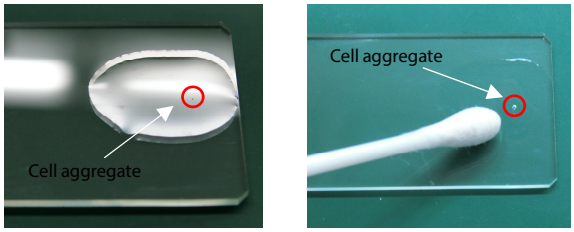
Fig. 2 Analysis by Cell3iMager duos

Table 3 Average Size of the Cell Aggregate Samples

HEK293	231.31 µm dia.
iPS Cell A	243.13 µm dia.
iPS Cell B	225.59 µm dia.

■ Test Method

Fig. 3 shows the pretreatment method. The cell aggregates were instilled one at a time on glass slides using a micropipette. During the preparation of the samples, droplets would cover cell aggregates. If tested in this status, the droplet would come in contact with the plate interfering with the test. To avoid that, the droplets were wiped away gently using paper towels and cotton swabs, then the compression test was performed within 30 seconds using the MCT-510.



a. Cell Aggregate after Instillation **b. Removal of Surplus Moisture**

Fig. 3 Pretreatment Method

Table 4 shows the MCT-510 test conditions. Fig. 4 shows the MCT-510 and a schematic of the testing. With the MCT-510, compression tests can be performed on one particle at a time, and the compression process can be checked on screen using the side observation kit.

The formula for calculating the deformation strength is shown below. The value measured by Cell3iMager duos was used for the particle size d. Normally, an optical microscope equipped with an MCT-510 is used to measure the particle size. With the cell aggregates used in this test, however, there was the risk of deformation due to drying and to the flexibility of the aggregates themselves, so the value measured by Cell3iMager duos was used.

$$\sigma_{10\%} = \frac{F_{10\%}}{A} \quad \cdots (1)$$

$$A = \frac{\pi d^2}{4}$$

$\sigma_{10\%}$: Deformation strength for a 10 % compression displacement of particle size (Pa)
$F_{10\%}$: Force for a 10 % compression displacement of particle size (N)
A : Typical surface area (m^2) (Area equivalent to a circle, found from the particle size measured before compression)
d : Particle size (m)

Table 4 Test Conditions for the MCT-510

Testing Machine	:	MCT–510
Top Compression Indenter (µm)	:	500 dia.
Test Mode	:	Flexible measurement mode
Test Force (mN)	:	400
Loading Speed (mN/sec)	:	3.8736
Measurement Cycles (No.)	:	5

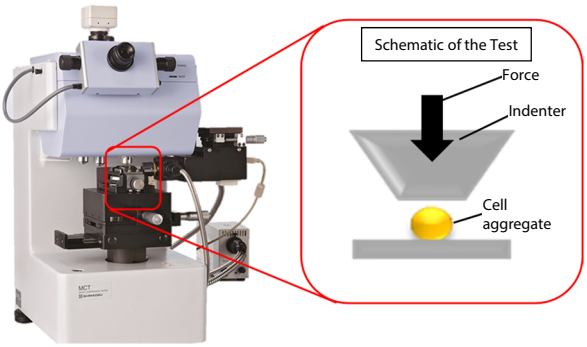


Fig. 4 MCT™-510 and Testing Schematic

■ Test Results

Table 5 shows the test results (average at five points). During the test no clear fracture points were detected. To define the particle strength, a $\sigma_{10\%}$ deformation strength was employed, and it was calculated from the force at a 10% deformation of the particle. The order of deformation strengths is as follows. A significant difference was found between the HEK293 and iPS cell A, and a difference was also found between the differently derived iPS cells.

HEK293 > iPS cell B > iPS cell A

Fig. 5 shows the force-displacement curve, and Fig. 6 shows cell aggregate compression by the MCT-510. The change in force was minimal in the region of about 20 to 60 µm displacement for HEK293 and about 20 to 40 µm for the iPS cells, and the most significant particle deformation occurred in these regions.

Table 5 Test Results

Sample	HEK293	iPS Cell A	iPS Cell B
Average Size (µm)	271.78	243.13	225.59
Deformation Strength $\sigma_{10\%}$ (MPa)	1.91	1.26	1.77

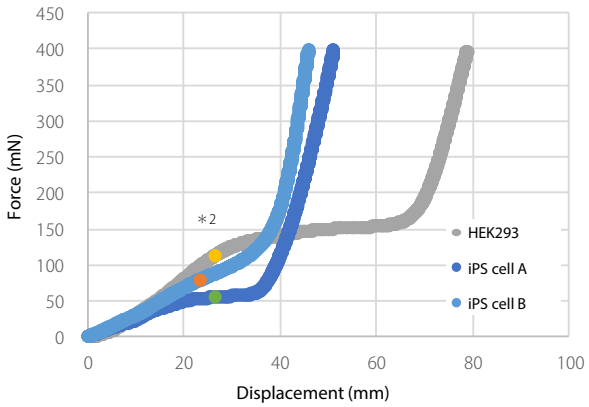
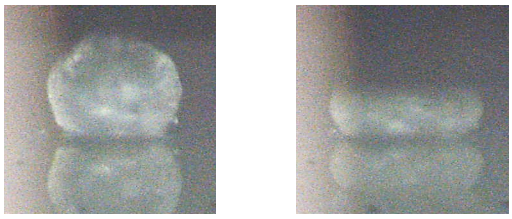


Fig. 5 Force-Displacement Curve

*2: The dots indicate 10 % deformation of the particle size.



a. Before Compression **b. After Compression**

Fig. 6 Compression of Cell Aggregates by the MCT-510 (Side Observation Kit)

■ Summary

Quantitative quality control of cells and tissues is required to make regenerative medicine more widespread. This article presents an example of a quantitative evaluation using deformation strength, one of their mechanical characteristics.

Cell aggregates are flexible and generally difficult to evaluate. However, compression test results obtained using the Shimadzu MCT-510 indicated the possibility of identifying the characteristics of each cell type. Further, even with the same cell type, differences were detected depending on their derivation, making this test of interest in quality control processes for cells and tissues. The MCT-510 micro compression testing machine is effective as a new evaluative index of flexible and easily deformed samples.

First Edition: Mar 2021

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