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Application News

No. S38

Scanning Probe Microscope

Observation and Measurement of Cells with SPM (I): Observation of iPS Cells and HeLa Cells

Outline

Astonishing progress has been achieved in regenerative medicine using iPS cells in recent years, and clinical research has also been reported. In these efforts, the characteristics of the iPS cells, such as their colony shape and proliferation rate, differ depending on the cell line derivation and culture method (Fig. 1), and it has been pointed out that iPS cells may form cancer cells, depending on the case. It can be inferred that differences in iPS cells, which can be called as individuality, are one factor that controls differentiation into diverse kinds of cells. Elucidation of this individuality has the potential to become an innovative technology in regenerative medicine. However, there are many unknowns concerning what imparts individuality, and this has become an obstacle in the use of iPS cells.

Therefore, we attempted cell shape observation with a scanning probe microscope (SPM), which has not been reported previously. The specimens used in this study were undifferentiated iPS cells and, as an extreme opposite type, cancerous HeLa cells.

This study clarified the fact that HeLa cells are dome-shaped, whereas iPS cells are flat and intercellular adhesion takes the form of a network structure.

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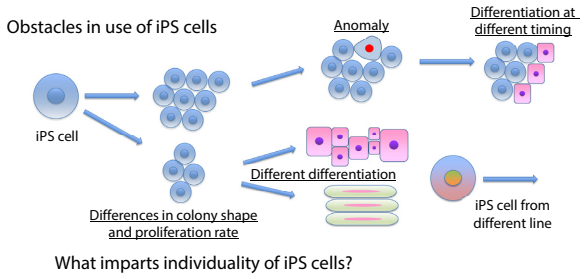

Fig. 1 Obstacles in Use of iPS Cells

Shape Images of HeLa Cells and iPS Cells

Fig. 2 shows SPM shape images (a) HeLa cells and (b) iPS cells. The corresponding phase difference images observed with an optical microscope are shown in (c) and (d), respectively. In the SPM shape images, bright areas are convex and dark areas are concave. The cross-sectional shape profiles at the positions indicated by the arrows in the figure are shown in (e) and (f). The individual HeLa cells are observed as dome-shaped, but in contrast, the iPS cells are relatively flat. Focusing on the boundaries between pairs of cells, those between HeLa cells are concave, but those between the iPS cells are convex and appear to form a network. It is thought this indicates a difference in intercellular adhesion, and suggests that the adhesion between the HeLa cells is weak, whereas that between iPS cells is strong.

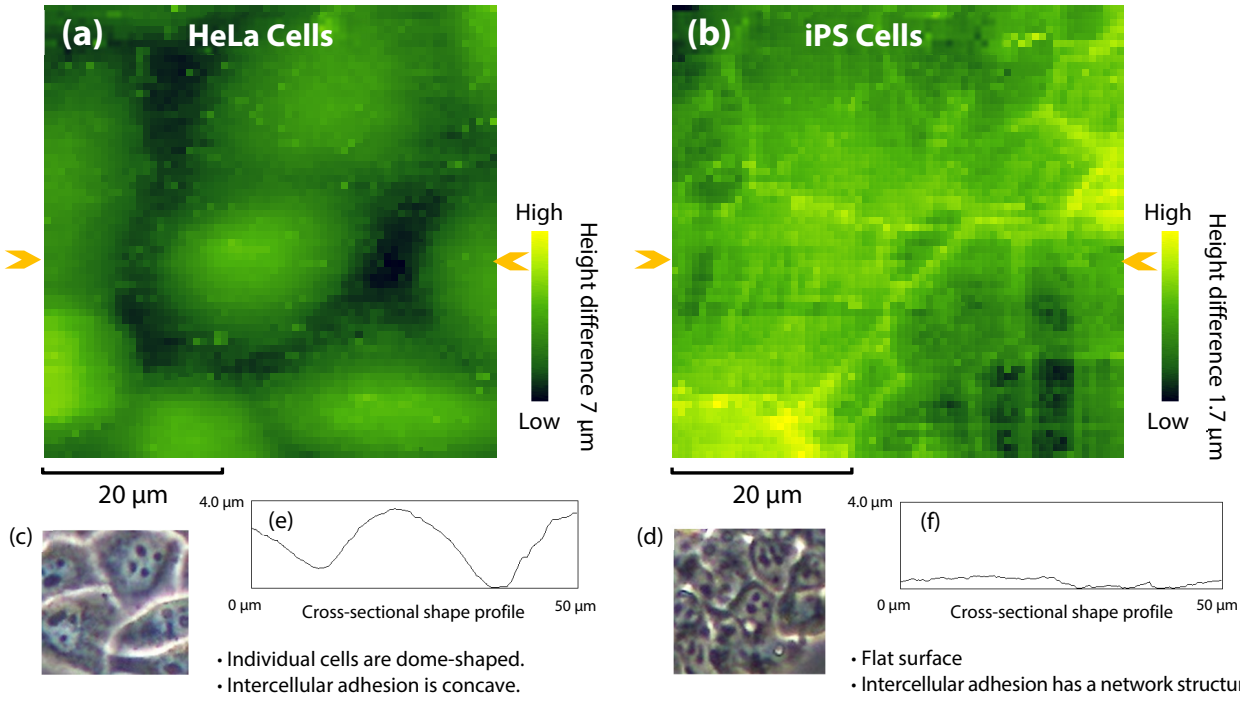

Fig. 2 Shape Images of HeLa Cells and iPS Cells

Cell Observation with SPM

Fig. 3 shows the configuration of the SPM, and Fig. 4 shows the configuration for observation in a solution. Although the SPM is different from optical microscopes and electron microscopes in that a beam and lens are not used in observation, its resolution is comparable to that of a transmission electron microscope (TEM). With the SPM, it is possible to acquire the shape of a specimen by tracing the specimen with a fine needle (probe) and detecting the minute force acting between the probe and the specimen as deflection of the cantilever.(1) In the contact mode and dynamic mode, which are conventional observation methods, the shape image is acquired by scanning the specimen surface in the horizontal direction, but in the case of soft specimens with large surface irregularities, like cells, the cell surface was scratched and it was difficult to obtain a normal shape. As a solution to this problem, force curve measurement was used. Fig. 5 shows a diagram explaining the force curve measurement method.(1) In force curve measurement, force is plotted while changing the distance between the probe and the specimen.(2) (3) (4) Because this operation does not involve horizontal scanning, soft specimens with large irregularities can also be observed without scratching the cell. In this example, the pressing force (repulsive force) on the cells was 2.5 nN.

After mapping measurement of the measurement area at 64 × 64 points, a shape image was formed from the acquired volume data. The cantilever used in this observation was an OMCL-TR800PSA manufactured by Olympus Corporation and had a spring constant of 0.15 N/m. The measurements were carried out with the cells in a live condition in a culture solution.

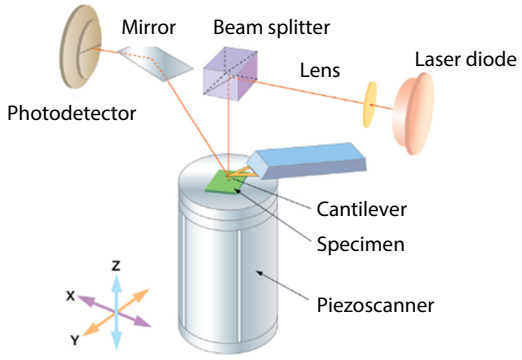

Fig. 3 Configuration of SPM

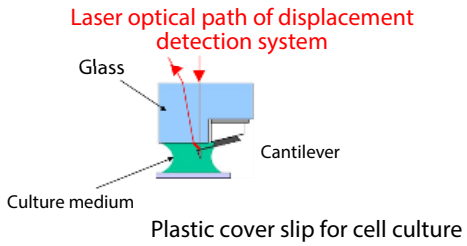

Fig. 4 Configuration of Observation in Solution
Specimens were observed in a solution with this configuration.

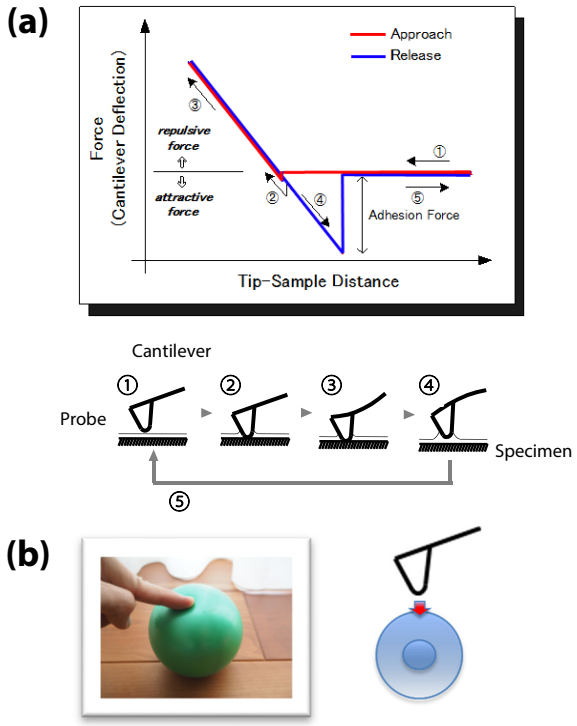

Fig. 5 Explanation of Force Curve Measurement

(a) Force applied to the probe is measured while changing the probe-specimen distance.
Pressing (Approach) is stopped when the force reaches 2.5 nN, and the probe is retracted (Release). A shape image is formed by mapping the Z-positions at which force reaches 2.5 nN.
(b) Feeling the shape of a ball by pressing it with a finger is an easy-to-understand image.

Conclusion

The shapes of HeLa cells and iPS cells in a living condition could be acquired by SPM measurement. These SPM measurements clarified the fact that HeLa cells are dome-shaped, and iPS cells are flat and intercellular adhesion takes the form of a network structure. It is considered possible that this network structure of intercellular adhesion has some kind of influence on maintenance of the undifferentiated state and the pluripotency of iPS cells.

<References>

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