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Differentiation of C2C12 myoblast cells quantitatively assessed by change in acoustic properties using ultrasound microscopy

超音波顕微鏡により取得した音響特性にもとづく C2C12 筋芽細胞の分化過程の定量評価

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1. Introduction

Ultrasonic microscope can observe biological matters quickly and non-destructively without chemical staining. In addition quantitative evaluation may be available by means of elastic parameters[(1)(2)].

In this study, biological cells, C2C12 myoblast were observed and assessed. This type of cells, after being induced with differentiation, will grow into contactable muscle fibers or myocells. The assessment for the differentiation process from acoustic characteristics would be applied in the process of regenerative medicine as one of the condition in cells monitoring method. This series of studies is conducted to determine whether quantitative ultrasonic microscopy can monitor cell differentiation.

In this paper, the result of acoustic observation will be described in terms of local characteristic acoustic impedance of the cytoskeleton in individual cell, in the process of differentiation.

2. Observation system

C2C12 myoblasts used in this study are clones of mouse. These cells remain undifferentiated at the initial expression of heart and skeletal muscles. The cells were cultured in a culture vessel that is made of polystyrene films with 50 μm in thickness and 3.2 cm^2 in culture area. The pulsed ultrasound (central frequency: 300 MHz) was focused at the interface between a cell and the film substrate. The reflection is received and interpreted into characteristic acoustic impedance. A 2D acoustic impedance profile was obtained by mechanical scanning[(1)].

The acoustic impedance of the target substance, Z_{target}, is determined by comparing the reflections from the reference material (culture liquid) and the target as:

$$Z_{\mathrm{target}} = \frac{1 - \frac{S_{\mathrm{uarget}}}{S_{\mathrm{ref}}} \cdot \frac{Z_{\mathrm{sub}} - Z_{\mathrm{ref}}}{Z_{\mathrm{sub}} + Z_{\mathrm{ref}}}}{1 + \frac{S_{\mathrm{uarget}}}{S_{\mathrm{ref}}} \cdot \frac{Z_{\mathrm{sub}} - Z_{\mathrm{ref}}}{Z_{\mathrm{sub}} + Z_{\mathrm{ref}}}} Z_{\mathrm{sub}}$$

where S_{ref} and S_{turget} are the acoustic impedances of the film substrate (2.46 MNs/m^3) and cultured lipuid (1.52 MNs/m^3), respectively.

3. Results and Discussion

C2C12 myoblast cells were subjected to differentiation induction for 6 days. **Fig. 1(a), (b)** show the physiological observation of C2C12 myoblast cells before and after differentiation. **Fig. 1(c), (d)** show the 2D acoustic impedance profiles in the field of views corresponding to Fig. 1(a), (b), respectively.

The shape of cells did not undergo much change before and after differentiation in the observation using optical microscope. However, the acoustic impedance at some specific points apparently increased after differentiation.

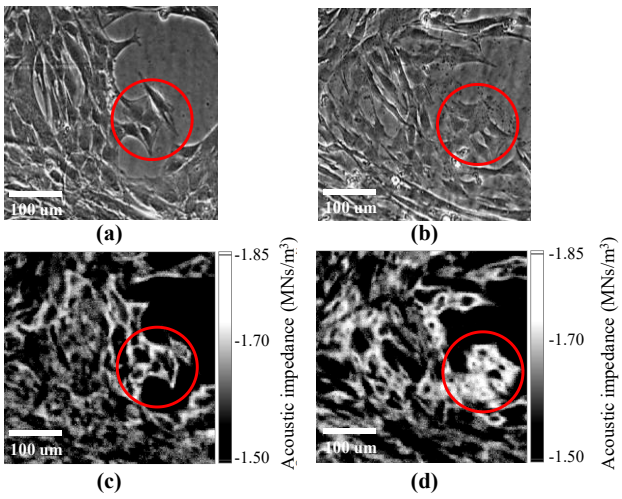

Fig. 1　Physiological observation images and acoustic impedance profiles of C2C12 myoblast cells. Undifferentiated cells ((a), (c)) and differentiated cells after 6 days ((b), (d)) are shown.

The intracellular structure seems to have changed without the changing of shape after the differentiation. Corresponding to the change in cytoskeletal

protein, the high acoustic impedance around the nucleus would increase. Hence, we propose a method to assess the spatial spreading of cytoskeletal region by means of acoustic impedance profiles.

Fig. 2(a) shows an example of viewport extracted from the acoustic impedance profile. The size of the viewport was arbitrary determined. Assuming that the center is the nucleus, a radial straight line with the radius *R* was drawn starting from the center. The highest point in acoustic impedance along this line was interpreted into the distance to the cytoskeleton from the nucleus. This number was averaged with different angles (θ) indicated in Fig. 2(a). The extracted parameter would indicate the scale of the cytoskeleton.

Subsequently, the half width was determined for each acoustic impedance distribution along the straight line (**Fig. 2 (b)**). This half width would be taken as a parameter indicating the spread of the cytoskeleton. The straight line was rotated for 360 deg , and the maximum acoustic impedance and half width were determined for every 1 deg. The parameters were averaged after taking these numbers for each radial line

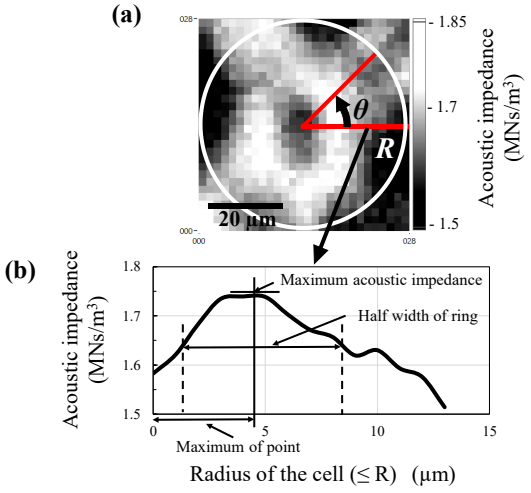

Fig. 2 Evaluation for high-acoustic-impedance ring : (a) An example of extracted viewport. (b) Acoustic impedance distribution along an angular straight line in (a).

Fig. 3 shows the result of the half width versus the maximum acoustic impedance for 57 cells (27 cells for day 0, 30 cells for day 6). By Student t-test, the maximum acoustic impedance and half width around nucleus cell had a significant difference before and after differentiation.

This difference suggests that the volume of cytoskeleton inside the cells would increase after the differentiation, due to notable expression of cytoskeletal protein. It is considered that these cells that have such characteristic would be differentiated into myocells.

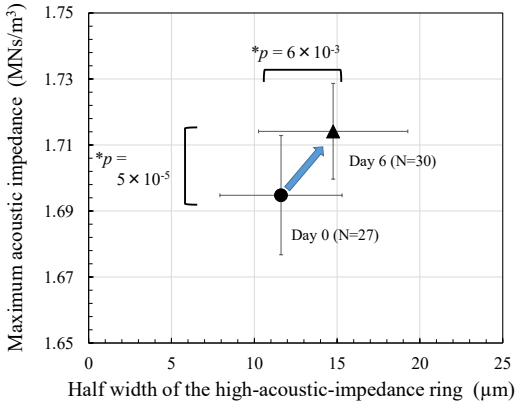

Fig. 3 Relation between half width of the high-acoustic-impedance ring and the maximum acoustic impedance in the corresponding ring. 57 cells (27 cells for day 0, 30 cells for day 6) were subjected to the analysis.

4. Conclusion

Differentiation process of C2C12 cells was assessed by means of acoustic microscope. Ultrasonic beam was transmitted across a plastic film substrate on which cells were cultured, and the reflection was interpreted into acoustic impedance. This quantitative observation is noninvasive to cells, as well as making it possible to continuously monitor the change in acoustic properties through the differentiation process.

By observation using optical microscope, it was found that there was no significant change in cell shapes before and after the differentiation. However, a notable difference was seen in acoustic impedance.

we propose a method to assess the spatial spreading of cytoskeletal region by means of acoustic impedance profiles, and evaluated 57 cells before and after differentiation. It was suggested that the volume of cytoskeleton inside the cells would increase after the differentiation, due to notable expression of cytoskeletal protein.

Further consideration will be needed to yield any findings about the cultural condition of cells and the influence of days elapsed.

References

1. Naohiro Hozumi et al. 2013 Joint UFFC, EFTF and PFM symposium, 1212-1215(2013)
2. Agus Indra Gunawan et al. Ultrasonics 61, 79-87(2015)