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Subcellular Observation of C2C12 Myoblast Differentiation Using Ultrasonic Microscope

超音波顕微鏡を用いた C2C12 筋芽細胞の分化過程の細胞内変化の観察

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1. Introduction

Two-dimensional acoustic impedance imaging is useful for observation of elasticity on living organs with no invasion [1][2]. The high-resolution acoustic impedance microscopy with 320 MHz transducer could visualize intracellular conditions of cultured cells, especially the distribution and condition of their cytoskeletons. Cytoskeleton is a complex structure like meshwork of protein fibers to exist in the cell. In particular, actin filament (F-actin) is closely related to cell formation, proliferation and differentiation. F-actin is repeating dynamically between polymerization and depolymerization in living cells, and stabilized with some actin-binding proteins.

Murine C2C12 myoblasts, which are undifferentiated immature muscle cells, differentiate and fuse together to form mature muscle cells; myotubes *in vitro*[3]. During muscle cell differentiation, cytoskeletons would polymerize much and change their distribution.

In this study, we report the living cell observation of differentiating C2C12 muscle cells using the high resolution acoustic impedance microscopy and compare the physiological and biochemical observations.

2. Methods

2.1 C2C12 cell culture

C2C12 myoblasts (DS Pharma Biomedical Co.Ltd, Japan) were proliferated in Minimum Essential Medium Eagle with Hanks' salt (HMEM) supplemented with L-Glutamine, 10 % fetal bovine serum (FBS), some vitamins, amino acids, and 0.1 g/L Kanamycin. To induce differentiation, culture medium was replaced with serum-free medium. All cells were cultured and observed on the OptiCell™ (Thermo Scientific Nunc, USA). It is composed of polystyrene films of culture area 50 cm^2 and membrane thickness 75μm, and C2C12 myoblasts are injected and cultured in the space between two membranes. Some samples were treated with 25 μg/ml Cytochalasin B (CyB) [4].

To observe intracellular structure, the pulsed focus ultrasound (central frequency: 300 MHz) was transmitted and the reflection from the interface between cell and polystyrene film was received, and interpreted into characteristic acoustic impedance. The 2D profile of acoustic impedance was acquired by mechanically scanning the transducer.

Physiological and biochemical conditions were observed with pharmacological reaction and the expression of myotube dependent myosin protein (MY-32) using the immunocytochemistry.

3. Results and Discussion

Proliferated C2C12 cells on OptiCell™ were induced to differentiate with serum-free medium. Pre-differentiated C2C12 cells distribute randomly and show a few contractive reactions to 1 mM caffeine treatment, while differentiated C2C12 cells constrict frequently and express lots fast contractile proteins (**Fig. 1**). In fig. 1 b and e, dense blue squares show more frequent reactive areas. The differentiated cells express lots of MY-32 protein. These physiological changes would reflect the reconstruction of intracellular cytoskeletons.

The intracellular impedances in C2C12 cells were increased remarkably during their differentiation (**Fig. 2**). High impedance structure was distributed roundly in undifferentiated cells around cell nucleus and the impedance in undifferentiated cells was not changed. With the progress of differentiation, fibrous high impedance areas were observed and the intracellular impedance was increased. Intracellular high impedance areas would show the density of F-actin.

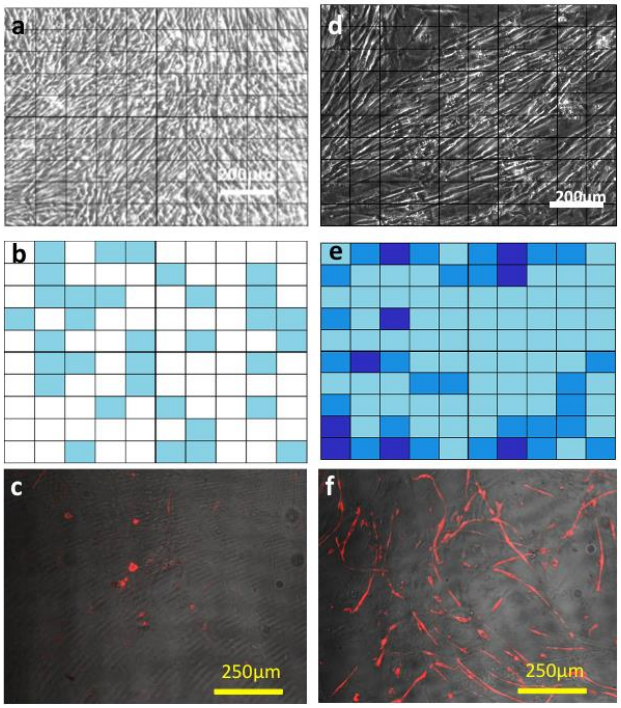

Fig. 1 Physiological and biochemical observation of C2C12 cell differentiation. Undifferentiated cells (a, b, c) and differentiated cells (d, e, f) are shown. a and d show phase contrast light microscopic images, and b and e show the contraction frequency to caffeine treatment in each culture. c and f show the expression of MY-32.

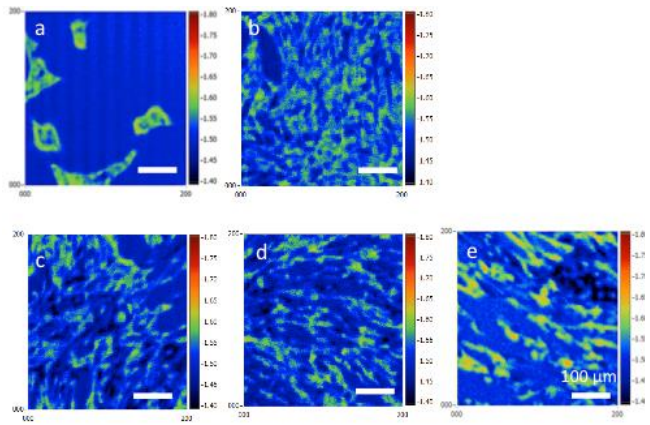

Fig. 2 Intracellular impedance of differentiating C2C12 cells. a, undifferentiated 2 day-cells, and b, undifferentiated 8 day-cells. In contrast, c, d and e are differentiated cells. c, 2 day, d, 4 day and e, 10 day after differentiation.

The treatment with CyB, which is F-actin depolymerizeing agent, shows the stability of F-actin. The impedance of C2C12 cells was decreased with CyB treatment, whereas the impedance of differentiated cells shows more resistance than undifferentiated cells (**Fig. 3**). In differentiated C2C12 cells, F-actin would be stabilized by some actin binding proteins.

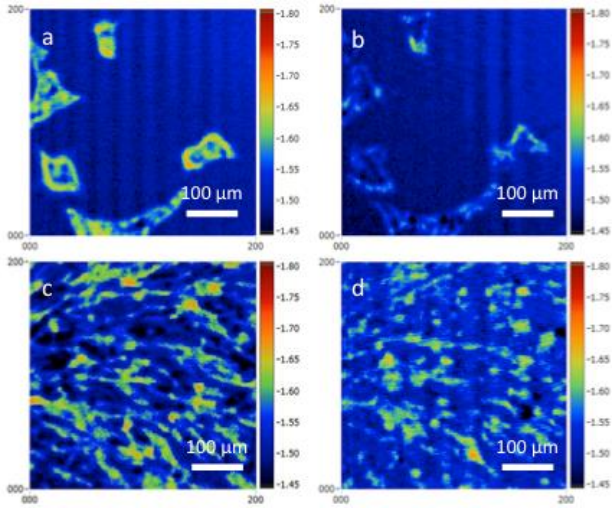

Fig. 3 The change of acoustic impedance with CyB treatment. a and b; undifferentiated C2C12 cells, and c and d; differentiated. a and c show pretreated images, and c and d treated images for 120 min.

4. Conclusion

We suggest that the acoustic impedance microscopy could detect the intracellular viscoelastic change during myotube differentiation. In differentiated C2C12 cells, intracellular impedance increased remarkably, and in parallel, they expressed mature-type protein, and showed the potential of contraction. High-resolution acoustic impedance microscopy was able to observe cytoskeletal change in living cells conveniently. We suggest that the acoustic impedance microscopy would useful to detect the intracellular conditions of artificial organs.

4. 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)
3. Burattini, S., et al. "C2C12 murine myoblasts as a model of skeletal muscle development: morpho-functional characterization." European journal of histochemistry: EJH 48.3 (2004): 223.
4. Flanagan, Michael D., and Shin Lin. "Cytochalasins block actin filament elongation by binding to high affinity sites associated with F-actin." *Journal of Biological Chemistry* 255.3 (1980): 835-838.