1P5-13

Proceedings of Symposium on Ultrasonic Electronics, Vol. 37 (2016)
16-18 November, 2016

Quantitative Evaluation Method of Differentiation Process in C2C12 Myoblasts Using Ultrasonic Microscope

超音波顕微鏡を用いた C2C12 筋芽細胞の分化過程の定量的評価法

Kyoichi Takanashi[1‡], Mamoru Washiya[1], Kazuki Ota[1], Naohiro Hozumi[1], Sachiko Yoshida[1] and Kazuto Kobayashi[2] ([1]Toyohashi University of Technology; [2]Honda Electronics Co., Ltd.)

高梨　恭一[1†]，鷲谷　衛[1]，太田　一樹[1]，穂積　直裕[1]，吉田　祥子[1]，小林　和人[2] ([1]豊橋技術科学大学, [2]本多電子株式会社)

1. Introduction

Ultrasonic microscope can observe biological matters quickly and non-destructively without chemical staining. In addition quantitative evaluation may be possible by using an elastic parameters image[1][2].

In this study, biological cells called as C2C12 myoblast were observed. This type of cells, after being induced with differentiation, will fuse and grow into the muscle fiber that has contractibility. If the differentiation process is revealed from the point of view of acoustic characteristics, it is expected to be applied as a condition monitoring method of cells in the process of regenerative medicine as well. The purpose of this series of studies is to evaluate the transition of cells that are creating three-dimensional structure in differentiation process by means of acoustic profile. In order to perform functional assessment of organs in the process of differentiation, it is highly required to determine three-dimensional structure as well as distribution in acoustic properties.

In this paper the result of acoustic observation will be described in terms of local characteristic acoustic impedance of cells, as well as of delamination from the culture substrate in the process of differentiation.

2. Methods

2.1 The observing system

Cells were cultured in a culture vessel that is made of polystyrene films with 75 μm in thickness. The pulsed focus ultrasound (central frequency: 300 MHz) was transmitted and the reflection from the interface between cell and film was received, and interpreted into characteristic acoustic impedance. The 2D profile of acoustic impedance was acquired by mechanically scanning the transducer.

2.2 The compensation of acoustic impedance

Fig. 1 shows a 2D acoustic impedance profile after differentiation. Cells are normally higher in acoustic impedance than culture liquid. However some points are displayed with significantly low acoustic impedance. By carefully observing the reflection waveform it was considered that two different reflections, that were making interfering. It was considered that at such a point the cell is not perfectly in touch with the film substrate. One component was very similar to the reflection from the culture liquid that was used as a reference waveform, and the other was considered as to be the reflection from the interface between the culture liquid and cell.

The reflection waveform at such a point was subjected to wave separation, by assuming that the each component of the waveform can be represented as

$$P_{(t)} = P_0 e^{\alpha(t-t_0)-\beta(t-t_0)^2} \cdot cos\{2\pi f_0(t-t_0)\} \quad (1)$$

where P_0 is the intensity, α is the attenuation constant, f_0 is the central frequency of the pulse, β is a parameter to define its width, and t_0 is delay time.

3. Results and Discussion

C2C12 myoblasts were subjected to differentiation induction for 10 days. **Fig. 2** shows the profile after compensation of the low acoustic impedance by using the above mentioned method. These acoustic impedance after successful compensation were 1.65 to 1.75 MNs/m^3.

It is suggested that changing the elastic force works under the cultural conditions[3][4]. **Fig.3** shows transition of the ratio of high acoustic impedance (>1.68 MNs/m^3) area to total cellular area after compensation. Each point shows the area ratio and standard deviation of the eight samples. In this graph, the area ratio of cells is prone to be decreasing. In the process of differentiation it is supposed that actin fibers that composes cytoskeleton once depolymerize in order to make cellular fusion easy. The reduction of the area ratio would indicate that

actin molecule / fiber had decomposed or packaged for differentiation to muscle cell.

Fig. 4 shows the distribution of the gap of cells from the culture substrate. This gap was confirmed, by means of acoustic analysis, as to be kept at constant during the process of differentiation due to pseudopodium adheres to the film substrate.

Therefore, it is assumed that the organization of cells is proceeding without changing component in the cells.

4. Conclusion

Differentiation process of C2C12 cells was assessed by means of acoustic microscopy. The ultrasonic beam was transmitted across the plastic film substrate on which cells were cultured.

This way of quantitative observation is specified as to be perfectly non-invasive to cells, as well as making it possible to continuously monitor the change in acoustic response through the differentiation process.

Along with the differentiation process, change in section of cytoskeleton was seen, suggesting de-polymerization and polymerization of actin fibers.

Waveform analysis made it possible to assess the delamination gap between the cell and film substrate that is also a factor to monitor the degree of differentiation. The present result suggested that the intracellular differentiation to muscle cell process can be evaluated by the transition of the acoustic assessment.

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)
3. Satoru Kidoaki, Takehisa Matsuda, 2007 Wiley Periodicals, Inc. J Biomed Mater Res 81A: 803-810(2007)
4. S. Kidoaki et al. Biomechan Model Mechanobiol 5, 263-272(2006)

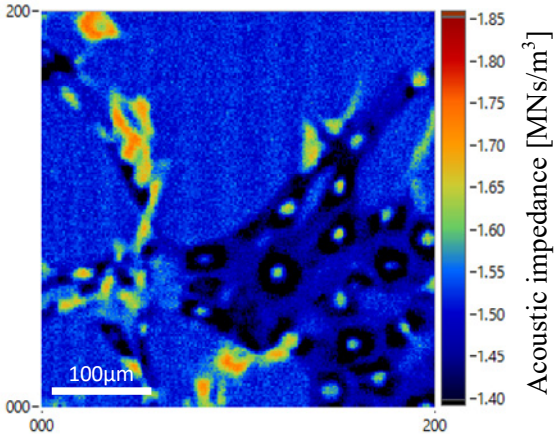

Fig.1 2D acoustic impedance profile

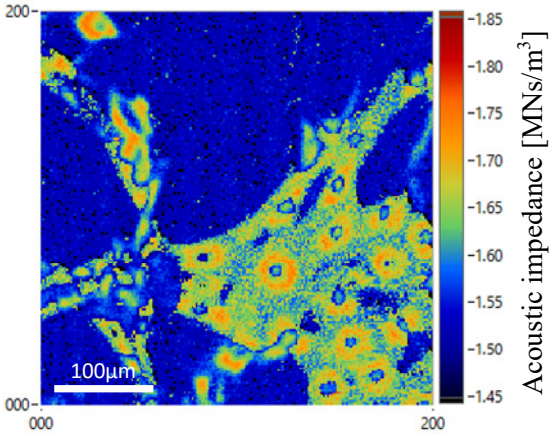

Fig.2 2D acoustic impedance profile after compensation

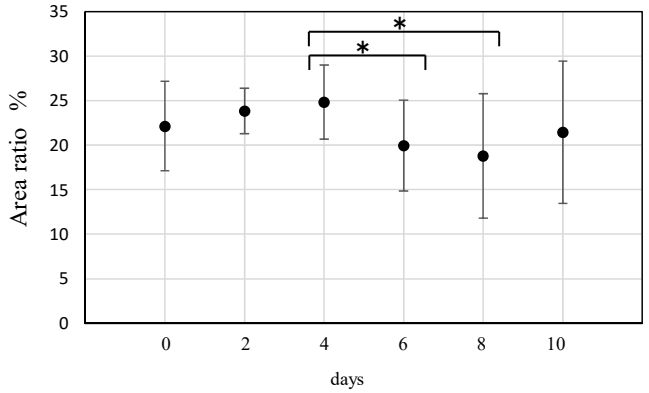

Fig.3 The ratio of the high acoustic impedance ($\geq$1.68 MNs/m^3) area to total cellar area in the 2D profile (*p = 0.07)

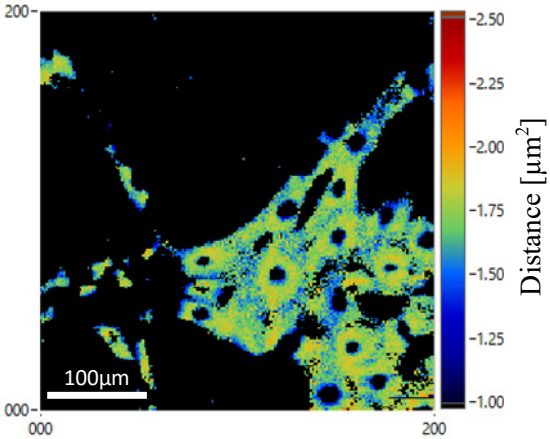

Fig.4 Distribution map of the delamination gap