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Acoustic impedance evaluation of myoblasts for quantitative diagnosis of sarcopenia

サルコペニアの定量診断にむけた筋芽細胞の音響インピーダンス評価

Ryoya Hashimoto[1†], Hitoshi Maruyama2[2], and Tadashi Yamaguchi[3*] ([1]Grad. School Sci. Eng., Chiba Univ.; [2]Grad. School Med., Juntendo Univ.; [3]Center for Frontier Medical Engineering, Chiba Univ.)

橋本 諒哉 [1†], 丸山 紀史 [2], 山口 匡 [3*] ([1] 千葉大学院 融合理工学府; [2] 順天堂大院医学研究科; [3] 千葉大 CFME)

1. Introduction

Sarcopenia is defined by loss of muscle mass and strength and frequently associated with diabetes and liver disease, which are major chronic diseases in the elderly. It is expected that the number of patients with chronic diseases complicated by sarcopenia will increase with the aging of the population, and early recognition of changes in the muscular composition is important for extending healthy life expectancy. However, since muscle is an organ that is extremely difficult to extract histological examination by sampling, it is required in clinical environment to introduce a simple and noninvasive evaluation method. Therefore, as a quantitative diagnostic method for sarcopenia, we focused on an ultrasonic diagnostic method that focuses on muscle tissue properties, that is, muscle fibrosis and fatification, unlike conventional evaluation methods focusing on muscle mass and muscle strength.

In this study, we attempted to evaluate the acoustic properties of myoblasts, which are the simplest structures of muscle tissue, using a scanning acoustic microscopy (SAM) equipped with a single element transducer with center frequency of 250 MHz.

2. Materials and Methods

2-1. Materials

In this study, we used myoblasts, which were primary cultured from around the gluteus maximus muscle of ICR mice 2 to 4 days old, as the scan target. The myoblasts were incubated in a flask containing a medium for growth (DE+10 %FBS) in an incubator, temperature of 37 °C and CO2 concentration of 5 %, for at least 1 day. After that, those were seeded on dishes made of polystyrene thin film with thickness of 50 μm and filled with mediums for differentiation (DE+2 % Horse Serum), and incubated again in an incubator, temperature of 37 °C and CO2 concentration of 5 %, for at least 8 hours to attach the myoblasts to the substrate. After induction of differentiation, the myoblasts can be cultured for about one week, and during this period, those were maintained in an incubator, temperature of 37 °C and CO2 concentration of 5 %, as much as possible to avoid unnecessary influence on them.

2-2. System

A SAM (modified AMS-50SI, Honda Electronics Co., Ltd, Japan) equipped a 250 MHz center frequency transducer (Fraunhofer IBMT, St. Ingbert, Germany) with the spatial resolution of 7 μm was employed for analyzing the acoustic impedance of the cultured myoblasts. The myoblasts were placed in a chamber (C-091A, BLAST Inc, Japan) where the temperature and CO2 concentration can be controlled during the scanning. The environment was maintained 37°C and 5% (**Fig. 1**). **Table 1** shows the characteristics of the transducer used in this study. RF echo signals in 2D plane could be acquired by scanning the transducer in X-Y direction. The acquired data points were always 300 by 300 points and the physical size of the final image was 300 μm × 300 μm (i.e., 1 μm step size). In order to reduce noise, the signal averaging was conducted (8 times). For a total of 6 days, at least 2 dishes and 6 ranges or more scanned. In addition, the dish used for scanning was not reused.

Table.1 TRANSDUCER SPECIFICATIONS

Center frequency	250 MHz
6 dB bandwidth	190 MHz
Membrane	ZnO
Aperture size	520 μm
Depth of focus	500 μm
Lens	Sapphire
Lateral resolution	7 μm

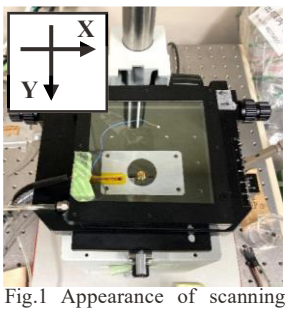

Fig.1 Appearance of scanning acoustic microscopy

2-3. Acoustic Impedance Analysis

The acoustic impedance was calculated based on the analysis of echo amplitude which was suggested by Hozumi [1][2]. In analysis of acoustic impedance, mediums for differentiation was used as a reference material, and polystyrene was used as a substrate with already-known acoustic impedance. The acoustic impedance of the mediums is calculated in advance to be 1.51 (±0.019) Mrayl, and that of polystyrene is 2.37 Mrayl. The target signal S_{target} (the reference signal S_{ref}) can be described as

$$S_{target(ref)} = \frac{Z_{target(ref)} - Z_{sub}}{Z_{target(ref)} + Z_{sub}} S_0 \qquad (1)$$

where S_0 is the transmitted signal, S_{target} (S_{ref}) is the signal from target (reference). The parameter S_{ref} is average of signals of other dish mediums. Z_{target} and Z_{sub} are the acoustic impedance of the target and substrate, respectively. Both S_{target} and S_{ref} are measurable parameters, however S_0 is not able to measure directly. Therefore, acoustic impedance of target Z_{target} is calculated as a solution of the simultaneous equations of S_{target} and S_{ref}, described as eq. 2.

$$Z_{target} = \frac{1 - \frac{S_{target}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}}{1 + \frac{S_{target}}{S_{ref}} \cdot \frac{Z_{sub} - Z_{ref}}{Z_{sub} + Z_{ref}}} Z_{sub} \qquad (2)$$

An appropriate threshold value was set for the entire scanning range of each data, and the average value and standard deviation were calculated for each scanning day.

3. Results and Discussion

Figure 2(a) shows an example of acoustic impedance image calculated from the maximum amplitude on each measurement point. Multiple myoblasts could be confirmed.

Figure 2(b) shows average and standard deviation of the acoustic impedance of myoblasts on each scanning day. It is shown that the acoustic impedance tends to be low (correlation coefficient: -0.6627) over time from attaching the myoblasts to the substrate. However, the difference in acoustic impedance is extremely small comparing with the difference of general tissues.

4. Conclusion

We confirmed that stable acoustic impedance analysis of fresh mouse myoblasts was possible using a scanning acoustic microscopy equipped a 250 MHz center frequency transducer and the chamber. However, in order to discuss relationship between acoustic characteristics and structure, it is necessary to measure with multiple modalities such as an optical microscope and a confocal microscope in addition to a scanning acoustic microscopy and compare the results in a one-to-one correspondence.

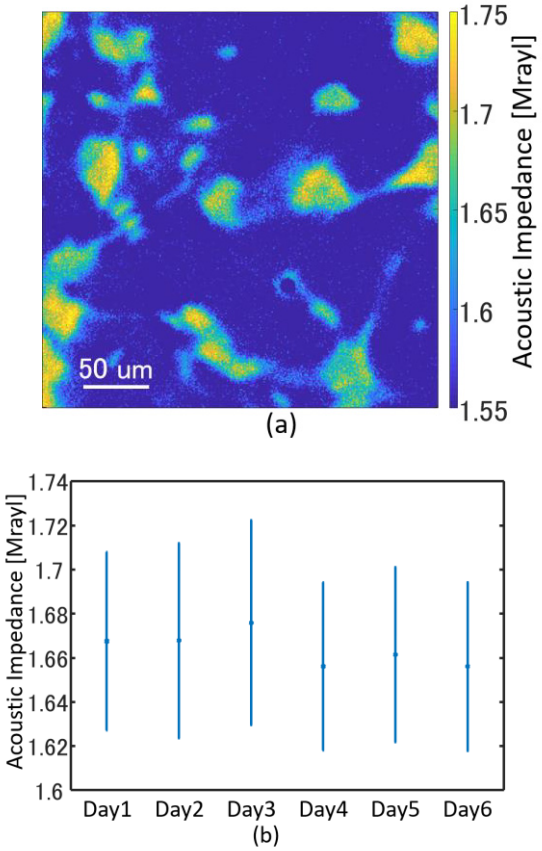

Fig.2 Acoustic impedance image of myoblasts on a dish (a) and average and standard deviation of impedance of myoblasts on each scanning days (b).

Acknowledgment

This work was supported by JSPS Core-to-Core Program, KAKENHI 19H04482, and the Institute for Global Prominent Research at Chiba University.

References

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†ryoya_hashimoto@chiba-u.jp, *yamaguchi@faculty.chiba-u.jp