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Three-dimensional evaluation of tissue degeneration derived from muscle diseases using acoustic impedance as an indicator

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1. Introduction

In recent years, many quantitative tissue characterization methods using sound waves have been developed. However, the resolution in the frequency range used in clinical practice is several hundred micrometers to several millimeters, and evaluation is performed in a state where multiple tissue structures are mixed together. In order to evaluate such complex conditions in more detail, ultrasound microscopy, which can analyze not only morphology but also acoustic properties, has been used to analyze the acoustic properties of biological tissues in two dimensions. Usually, two-dimensional acoustic impedance maps are obtained as information on the surface of the tissue to be evaluated, but for more accurate evaluation, analysis of the three-dimensional structure of the biological tissue is required. In this report, we investigated the acoustic impedance in the depth direction of the tissue to be evaluated.

2. Materials and Methods

2.1 Materials

Fifty-two week-old normotensive rats (WKY/Hos) (Hoshino Laboratory Animals, inc) were used for evaluation. The back muscles were removed from each rat, and the muscles were cut orthogonally to the muscle fibers in the short-axis plane for evaluation.

2.2 Acoustic impedance analysis

Acoustic impedance is the product of the intrinsic sound velocity and density of a material. In this study, the acoustic impedance Z_{target} of purified water, which is known, was calculated as the acoustic impedance Z_{ref} of purified water and that of the substrate (polystyrene) Z_{sub} can be calculated by the following equation.

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

S represents the signal intensity of each signal at an arbitrary frequency. S_o is a transmitted wave and cannot be measured directly, while S_{ref} and S_{target} are reflected signals from the purified water and the measurement sample, respectively, and can be measured directly.

2.3 Methods

An ultrasonic microscope system (AMS-50SI modified, Honda Electronics Co., Ltd, Japan) incorporating a 250 MHz ZnO transducer (Fraunhofer IBMT, St. Ingbert, Germany) with a spatial resolution of 7 μm in the aperture direction at the focus was used. The muscle cross section was placed on a 50 μm-thick polystyrene film dish, and the transducer was scanned two-dimensionally (1,200 μm × 1,200 μm) from the underside of the dish through purified water to collect 8-bit three-dimensional echo data (300 points × 300 points × 200 points) in depth direction. As shown in **Fig. 1**, the acoustic impedance was evaluated by taking the ratio of the reflection signal from the boundary between the dish and the sample to that from the dish and the reference material at each scan position. Here, the acoustic impedance of the reference material (ultra-pure water) and polystyrene film dish were set to 1.50×10^6 and 2.37×10^6 kg/m^2/s, respectively, based on the results of the preliminary evaluation[1].

Tab. 1 Characteristics of transducers.

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

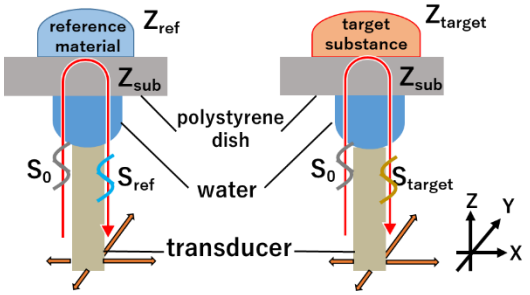

Fig.1 Methods of acoustic impedance evaluation.

Generally, acoustic impedance is obtained as information on the surface of the object to be evaluated. However, if echoes are generated at deeper locations (in the direction of ultrasound propagation) that are stronger than those at the surface, the acoustic impedance at the location where the echoes are generated is evaluated. By evaluating the acoustic impedance from the echoes at each depth, it is possible to understand the relationship between the internal structure and acoustic properties of biological tissues. Therefore, as shown in **Fig. 2**, envelope detection was performed on the received signal at all 300 points × 300 points in the three-dimensional echo data acquired in section 2.3, and logarithmic compression was performed on each envelope amplitude. For the obtained three-dimensional envelope amplitude data, acoustic impedance two-dimensional maps were created at each time (depth) every 0.5 ns with the measurement cross section (t = 0 ns) as the reference.

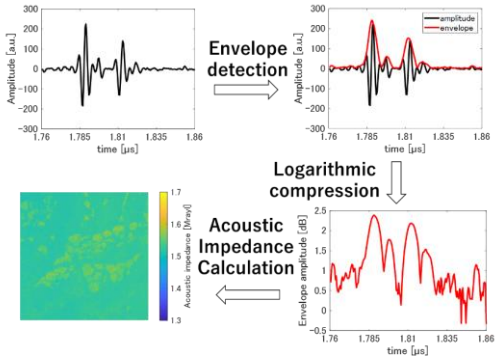

Fig.2 Calculation of acoustic impedance at each depth.

3. Results

Figure 3 shows two-dimensional acoustic impedance maps for each echo data at various depths. In the measurement cross section (z = 0 μm), structures that appear to be muscle fibers can be seen at high values of acoustic impedance. Similar muscle structures can be seen down to about z = 21 μm, which is relatively good data. However, it can be confirmed that the structure is difficult to recognize at deeper depths after that. This is a reasonable result in relation to the DOF of the transducers used for the actual measurements.

Since the target signal contains many signals, the acoustic impedance must originally be calculated taking waveform separation into account. If the ultrasonic beam has a sufficiently long depth of focus compared to the depth to be evaluated and can be approximated by a plane wave, the propagation path along the beam can be equivalently represented by a one-dimensional transmission line, so it is necessary to consider it as a collection of lossless transmission lines with different characteristic impedances[2]. However, it is possible to understand the apparent 3D impedance map corresponding to the internal microstructure of the sample using the simplified method described in this study.

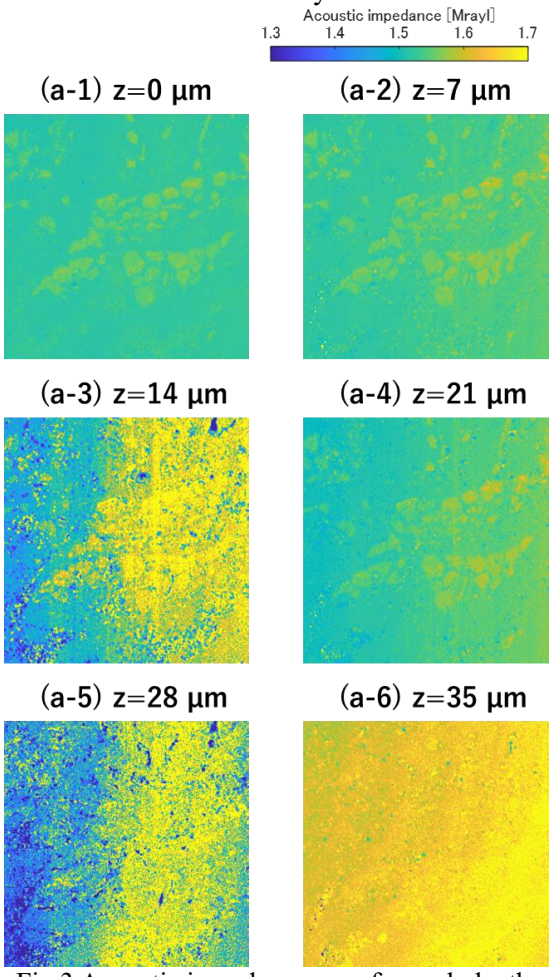

Fig.3 Acoustic impedance map for each depth.

4. Conclusions

By evaluating the acoustic impedance from the echoes at each depth, we investigated the relationship between the internal structure of biological tissue and its acoustic properties. Since it was confirmed that the data acquired by this system was relatively good in the depth direction, we will try to calculate the acoustic impedance by a method that takes the transmission path into account in the future.

Acknowledgment

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