1Pb5-3

Examination of temperature dependence in speed of sound evaluation of rat organs

Suguru Seto[1‡], Kazuki Tamura[2], Shinnosuke Hirata[3], Kenji Yoshida[3], and Tadashi Yamaguchi[3*], ([1]Grad. Sc. Sci. Eng., Chiba Univ.; [2]Hamamatsu Univ. School of Medicine.; [3]Center for Frontier Medical Engineering, Chiba Univ.)

1. Background

In our previous studies, we have confirmed that the post-fixation of flash-frozen tissue specimens for evaluating the speed of sound (SoS) of biological tissues using scanning acoustic microscopy (SAM) enables the evaluation of SoS as stable as that of paraffin-embedded specimens [1]. In addition, since the SoS varies depending on the temperature of the sample at the time of evaluation [2], there is a concern that care must be taken when evaluating fats, which have particularly pronounced thermal characteristics.

This research investigated the effect of temperature on the evaluation of the SoS in frozen specimens with post-fixation in several tissues with different properties, intending to evaluate the SoS of fat.

2. Materials and Methods

2.1 Target materials

The target organs of this study were the livers of 8-week-old normal, and 9-week-old fatty liver rat model (Slc:SD, male) shown in Fig. 1. In the first step, the target organs were removed from the rats, and multiple blocks were cut out from each organ. Then, each block was flash-frozen in liquid nitrogen via isopentane, thinly sliced to a thickness of 10 μm, and fixed on glass slides. The measurement samples were prepared by immersing thin sliced samples in half Karnovsky fixative.

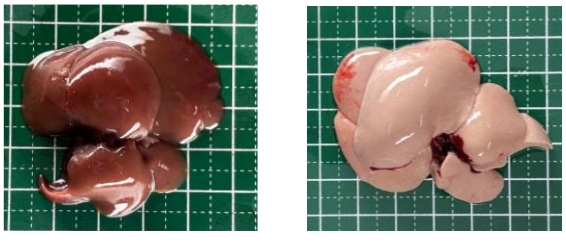

Fig. 1 Rat livers (a) normal, (b) fatty.

2.2 Data acquisition

A ZnO transducer (HT-400C, Honda Electronics) with the center frequency of 300 MHz and the spatial resolution of 7 μm was excited by a pulsar (GZ1120ME-03, GEOZONDAS) to observation. Three-dimensional (3D) RF echo data were acquired for a 6.4 mm × 8 mm area by scanning the transducer in two-dimensions on each sliced sample of target organs. At that time, the water temperature in the measuring vessel was kept at around 23 °C using thermostatic chamber, and then at around 36 °C for continuous measurement. The received signals were recorded using an A/D board (ATS9373, Alazartech) with a sampling frequency of 4.0 GHz and 12-bit quantization. After the staining of each sliced sample with the Hematoxylin-Eosin (HE) method, digital histological images (1 μm resolution) were acquired with a virtual slide scanner (NanoZoomer S60, Hamamatsu Photonics), and the histological structures were observed. Thin sliced samples of another section of each liver were stained with Oil red O to confirm the state of fat deposition.

2.3 Speed of sound analysis

The RF data of each scan line was up-sampled 10 times, and intensity map was acquired by normalizing with the maximum value of each scan line. In addition, linear tilt correction was performed for each scan line using the intensity and phase differences of the amplitude as indices to correct for the time difference fluctuations in the scanning directions. The echo signal components from the surface and back of the sliced sample were separated by eighth-order autoregressive model, and SoS was calculated from them using the echo signal of the manually selected glass area as a reference signal [3]. The average SoS of each sliced sample was calculated at the points where the echo signal intensity from the sample surface exceeded a threshold value (3.5 mV) in several regions of interest (ROI) of 400 μm × 400 μm settled on the SoS map. The position of ROI was selected as keeping correspondence with the digital histological images so that structures such as blood vessels and the sample edges are not included.

‡ s_seto@chiba-u.jp, * yamaguchi@faculty.chiba-u.jp

3. Results and discussion

Figures 2 (a-1), (a-2), and (b-1), (b-2) are SoS maps of normal and fatty liver measured at different water temperatures and show a 1.0 mm square area of maps to visually confirm the tissue structure. Figs. 2(a-3) and (b-3) show the HE-stained histological images corresponding to the SoS maps of each sample. In the SoS maps, outliers and areas where the echo signal intensity from the sample surface is below the threshold are masked in black.

It is assumed that the SoS evaluation corresponds to the tissue structure because the SoS map shows a texture corresponding to the histological image. Fig. 2 (b-3) shows cracks caused by ice crystals, but we observed the Oil Red O staining of the same liver, it was confirmed the presence of residual fat in the sample. However, there are no staining features indicating fat in the cracked area, suggesting a total loss of fat compared to the liver as it was in the body.

Figure 3 shows the results of SoS evaluation of each sample and all ROI at each temperature. The average SoS of the normal liver is about 20 m/s faster at the higher temperature, which is consistent with the results of the previous study. On the other hand, fat is known to slow down the SoS at higher temperature, but the results for fatty liver didn't show this tendency. The reason is assumed that the fatty liver has a different tissue structure from that of fat that exists alone, and that changes in the volume elasticity of the liver tissue were caused by the entry of fixative fluid into areas where fat has dissolved.

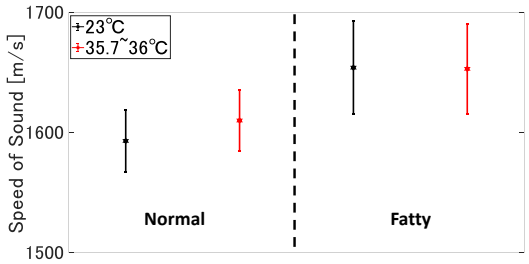

Fig. 3 Speed of sound of normal liver and fatty liver at different temperatures.

4. Conclusions

It was confirmed that the trends of the SoS change with temperature was different between normal liver and fatty liver. In the future, we will evaluate the SoS of fat alone and of thin sliced samples prepared by reducing ice crystals.

Acknowledgment

This work was partly supported by JSPS Core-to-core Program JPJSCCA20170004, and the Institute for Advanced Academic Research at Chiba University..

References

1. S. Seto et al., Proc. Acoust. J. Spr. in Japanese. 64-65, 2022.
2. H. Fukukita et al., BME Vol. 2, No. 3, 1988
3. N. Tanaka: IEICE Tech. Rep. 105 (2005) 21.

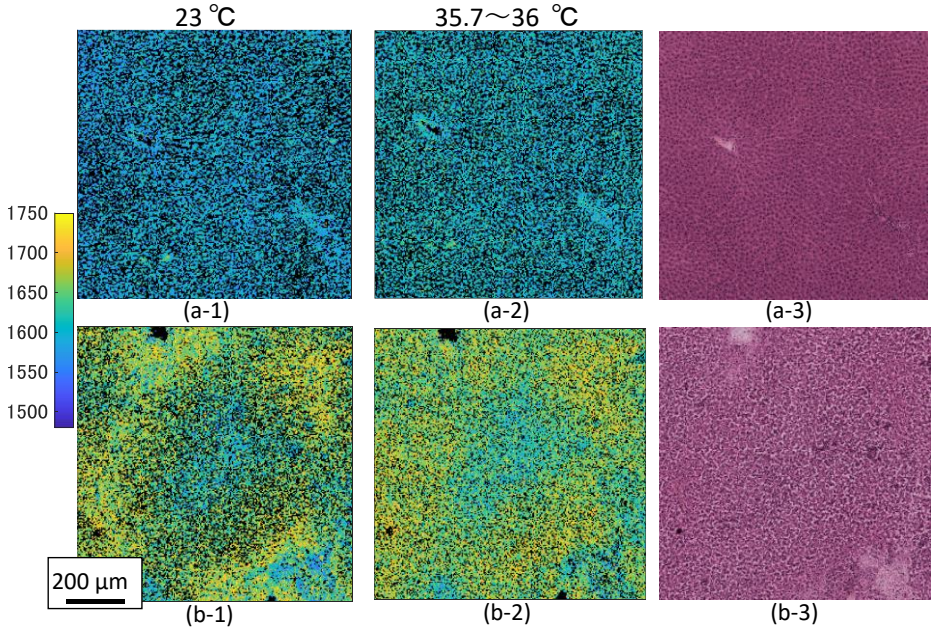

Fig. 2 2D speed of Sound maps at different temperatures and histological images of normal liver and fatty liver ((a-1, a-2, a-3):normal, (b-1, b-2, b-3):fatty).