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Ultrasound-spectroscopy imaging on human iPS cells for mechanobiology study

ヒト iPS 細胞の超音波スペクトロスコピーイメージングとメカノバイオロジー

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1. Introduction

Mechanobiology is defined as the study of mechanisms by which cells detect and respond to mechanical stimuli. Stem cells are considered to be more sensitive to the mechanical stimuli, and they are intensively studied in the mechanobiology field [1]. Stem cells, unspecialized cells, are capable of differentiation into any type of cells. It is now evident that a variety of biophysical and biomechanical cues play important roles in controlling stem cells behavior such as tissue morphogenesis [2], self-renewal [3], proliferation and differentiation [4]. However, many of these mechanisms remain unexplained.

In this research, we investigate the response of human iPS cells (induced pluripotent stem cells) to ultrasonic mechanical stimuli. The iPS cells are irradiated with ultrasound using a scanning acoustic microscopy (SAM), which also gives information of their mechanical properties through the acoustic imaging.

Various techniques have been used for mechanobiology study on single cells, including micropipette aspiration [5], laser tweezers [6], magnetometry [7] and atomic force microscopy [8]. These methods mainly provide information concerning cell adhesion and deformation related to the cell membrane and cytoskeleton. However, they are destructive because they require a mechanical contact with the cells by applying an external stress or strain. Furthermore, they cause macroscopic mechanical stimuli, and it is difficult to apply a local noninvasive mechanical stimulation with the previous methods. It is necessary to observe alive single cells toward deep understanding of cellular mechanical properties. The SAM provides us with information about sound velocity, density, stiffness, viscosity, and topography of the sample noninvasively. Therefore, it is a robust technique to investigate cellular mechanical response. No studies, however, appear for mechanobiology for iPS cells with SAM.

Here, we demonstrate the usefulness of the spectroscopic acoustic-imaging study using a 180-MHz SAM system, showing transition of cells movement and stiffness.

2. Experiment

The 180-MHz ultrasound waves were used for irradiating iPS cells that were immbolized on the plastic dish coated by substrate (Fig. 1). Then, the ultrasonic echoes from the cell surface and the dish surface were detected by the same probe, and their waveforms were acquired for making the spectroscopic acoustic images after the Fourier analysis. Furthermore, we irradiated a local point on the cell continuously with the high-frequency ultrasound and investigate the effect of the local mechanical stimulation on the stiffness and extension of the cell.

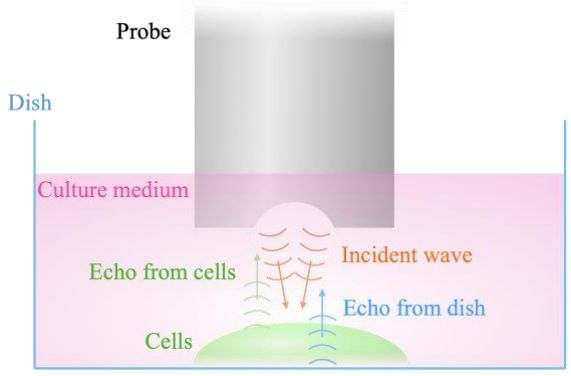

Fig. 1 Experimental setup.

3. Results and discussion

Figure 2 shows an example of the measured waveform, showing the echoes from the cell surface and the dish surface, which are composed of frequency components between 20 and 200 MHz with a maximum around 110 MHz.

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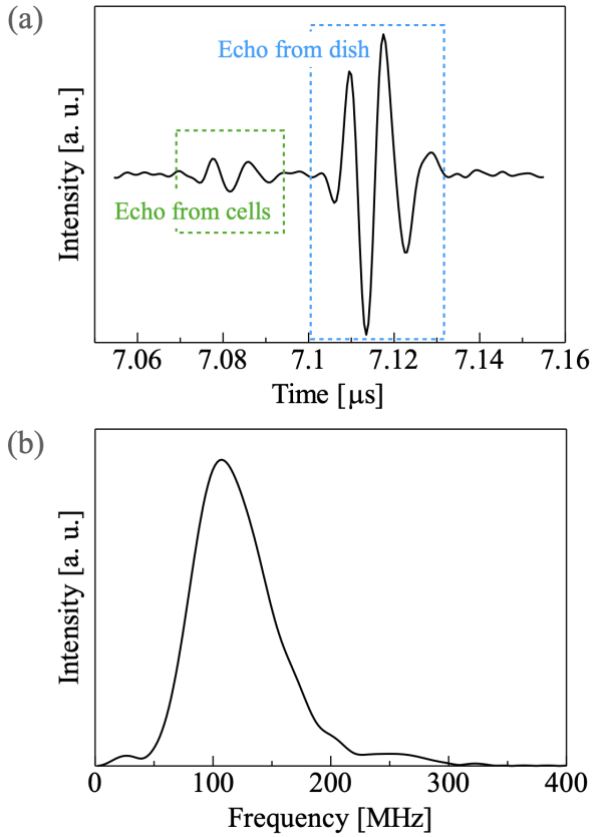

Fig. 2 (a)The waveform of the echo detected by the probe. (b) The waveform after the Fourier analysis of (a).

Figure 3 compares a phase-contrast-light microscope (PCLM) image with ultrasound images at specific frequencies. Each intensity was normalized by the maximum one and white area means high intensity. A higher-frequency image achieves better resolution as expected, but the S/N ratio deteriorates as the frequency increases: The 150 MHz image gives the clearest image in our system.

Interestingly, the image contrast is reversed at 80 MHz and higher frequencies, suggesting that the iPS cells preferably absorb ultrasound waves with frequencies higher than 80 MHz.

5.Conclusion

We established the spectroscopic ultrasound imaging system for cells, and succeeded in detecting ultrasonic echoes from the cell surface and the dish surface, for which the Fourier analysis was performed. Our results suggests that the iPS cell can absorb ultrasounds with specific frequency, which will be related with structures inside the cell. We will further study the effect of the local ultrasound stimulation on the cell extension and differentiation as well as the ultrasound absorption mechanism of the iPS cell.

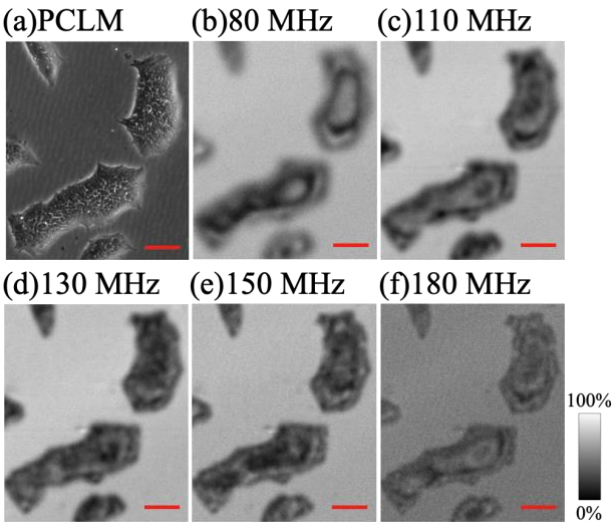

Fig. 3 (a) The PCLM image and the spectroscopic acoustic images at specific frequencies of (b)80, (c)110, (d)130, (d)150, and (e)180 MHz after the Fourier analysis. The scale bars indicate 100 μm.

References

1. B. Ladoux, and R. Mège, Nat. Rev. Mol. Cell Biol., **18**, (2017) 743.
2. C. Heisenberg, and Y. Bellaiche, Cell, **153**, (2013) 948.
3. E. Clayton, D. Doupé, A. Klein, D. Winton, B. Simons, and P. Jones, Nature, **446**, (2007) 185.
4. Y. Miroshnikova, H. Le, D. Schneider, T. Thalheim, M. Rübsam, N. Bremicker, J. Polleux, N. Kamprad, M. Tarantola, I. Wang, M. Balland, C. M. Niessen, J. Galle, and S. Wickström, Nat. Cell Biol. **20**, (2018) 69.
5. Yeung, and E. Evans, Biophys J., **1**, (1989) 139.
6. M. Sato, M. J. Levesque, and R. M. Nerem, Arteriosclerosis, **7**, (1987) 276.
7. M. Radmacher, M. Fritz, C. Kacher, J. Cleveland, and P. Hansma, Biophys J., **1**, (1996) 556.
8. M. Shibata, H. Watanabe, T. Uchihashi, T. Ando, and R. Yasuda, Biophys Physicobiol., **14,** (2017) 127.