Development of a high-frequency focused ultrasound system for applying noninvasively localized mechanical stimulation on cultured cells

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1. Introduction

Living cells sense various mechanical stimuli and respond to them by adjusting their tissue morphogenesis [1], self-renewal [2], proliferation and differentiation [3]. Especially, since stem cells are highly sensitive to mechanical stimuli, many studies have been conducted to clarify and control their behavior [4]. Stem cells, unspecialized cells, are capable of differentiation into any type of cells, and it is very important to control their differentiation paths. However, many of these mechanisms remain unexplained because of three main reasons given below.

First, 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]. However, they are destructive because they require a mechanical contact with cell. The cell-damaging prevents us from investigating pure effects of the stimuli and observing the effects for a long time exceeding the cell differentiation. Second, mechanical stimuli proposed in previous studies such as shear stress from liquid [9] or environmental vibration [10] fail to be localized to an intended location in the cell: Because these stimuli are spread throughout the cell, it is not clear whether the mechanical response of the cell is caused by stimulation of the entire cell or a specific part of it. This is one of the reasons why mechanisms are unclear. Finally, the power of stimulus cannot be controlled precisely in the previous methods.

In this study, we propose the high-frequency focused ultrasound to overcome these problems. Focused ultrasound is a non-invasive technique to mechanically stimulate a part in a cell, and its power for the stimulation can be controlled precisely by its frequency and amplitude. We originally develop the high-frequency focused ultrasound spectroscopic system for applying a localized mechanical stimulation in a single cell during the cell culture. It can be also used as the scanning acoustic microscopy (SAM), that provides us with information of their mechanical properties through the acoustical images.

Here, we apply the technique for human iPS cells (induced pluripotent stem cells) and investigate their response to the localized ultrasound stimulation. This is the first mechanobiology study of human iPS cells with a localized mechanical stimulation to systematically investigate its effects on the function of the cell.

2. Experiment

We used 150-MHz burst waves to make the localized mechanical stimulus in a human iPS cell. The duty cycle was 20 %. On the other hands, the 180-MHz low power ultrasound pulse wave was used to obtain the acoustic images. It was generated from the piezoelectric thin film deposited on the top surface of the acoustic lens and focused on the bottom surface of the cell by the acoustic lens (**Figure 1**). Then, the ultrasonic echoes from the cell surface and that of the dish surface were detected by the same probe, which were separately used for making the spectroscopic acoustic images after the Fourier analysis.

We stimulated the human iPS cell as shown in **Figure 2** adheared on the plastic dish coated by a substrate protein(iMatrix-511) for 1 h and monitored its effect on the stiffness and extension of the cell every 30 min. The experiment was conducted at 37 °C and 5 % CO_2 concentration.

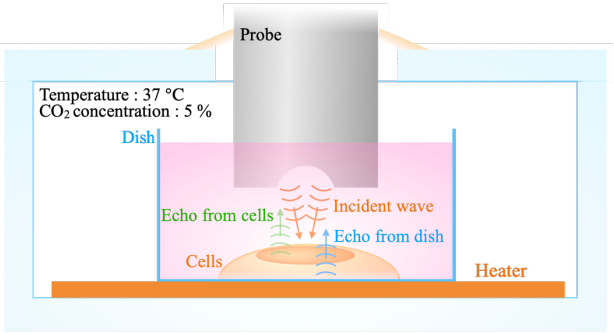

Fig. 1 Experimental setup for applying the local stimulus and obtaining the acoustic images.

3. Results and discussion

In order to confirm that sufficient ultrasound energy can be supplied to the cell, we irradiated the substrate protein layer with a 5- V_{pp} ultrasound, and the substrate layer was damaged as shown in **Figure 3.** We changed the applied voltage up to 5 V_{pp} and found a threshold voltage, over which the substrate

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protein layer is damaged, to be 2 V_{pp}. Therefore, we used the burst signal of 2-V_{pp} amplitude and 0.2-s duration for the mechanical stimulus. We can thus control the mechanical stimulus by controlling the driving voltage.

Figure 4 shows timelapse acoustic images created from the echo from the substrate. Each intensity was normalized by the maximum one and the white area means high intensity. The experimental system we constructed here allows us to monitor the living cells for longer than 24 hours. A higher-frequency image achieves better resolution as expected, but the S/N ratio deteriorates as the frequency increases. Interestingly, lower-frequency images represent nuclei more clearly, indicating that there is a specific ultrasound frequency for the nucleus to absorb the ultrasound energy. This means we can obtain the information of the effects on both cells structure and nuclei non-invasively by selecting the appropriate frequency.

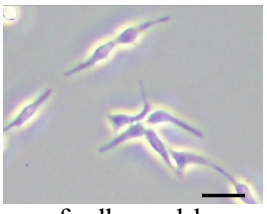

Fig. 2 Optical image of adheared human iPS cells. The scale bars indicate 50 μm.

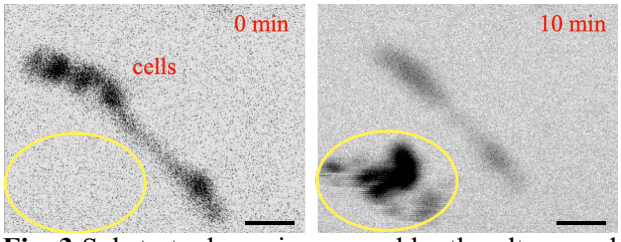

Fig. 3 Substrate damaging caused by the ultrasound stimulus of 5 V_{pp} input voltage. The scale bars indicate 50 μm.

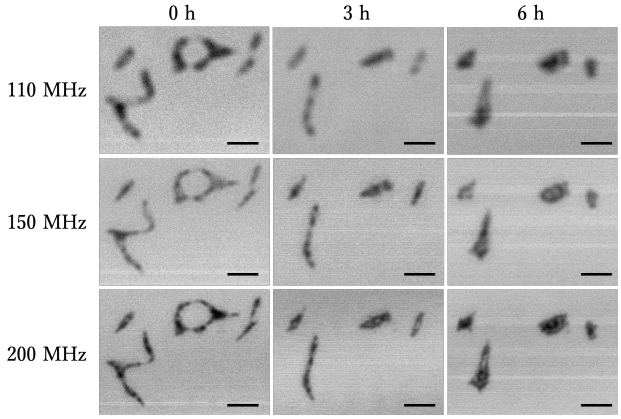

Fig. 4 The timelapse spectroscopic acoustic images for specific frequencies obtained during the cell culture. The scale bars indicate 100 μm.

4.Conclusion

We established a focused ultrasound spectroscopic technique for applying a non-invasive localized mechanical stimulation in a single cell to systematically investigate its effects on the function of the cell. We also made the spectroscopic ultrasound imaging for living cells at the same time and succeeded in monitoring them for longer than 24 hours.

Our results suggests that the human iPS cell show specific acoustic character when it is stimulated by localized mechanical stimulation. We will further study the effect of the local ultrasound stimulation on the cell extension and differentiation as well as the ultrasound absorption mechanism at a specific frequency of the human iPS cell.

References

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