Development of a focused ultrasound spectroscopic imaging system combined with optical imaging for applying mechanical stimulation on living 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)] 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, undifferentiated cells, are capable of differentiating into various cell types, and it is very important to control their behavior. However, many of these mechanisms remain unexplained because of three main reasons given below. First, the invasive problem: 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 invasive because they require a mechanical contact with cells. The cell-damaging prevents us from investigating only the effects of the stimuli and observing the effects for a long time exceeding the cell differentiation. Second, the macroscopic problem: mechanical stimuli proposed in previous studies such as shear stress from liquid[9)] and 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. Finally, the difficulty in controling the mecanical stimuli: the power of stimulus cannot be controlled precisely in the previous methods.

Therefore, for a deeper understanding of mechanobiology, precisely controlled mechanical stimuli must be applied noninvasively and locally, and their effects on cells should be systematically investigated over a long period of time. Of the methodologies currently available, only high-frequency focused ultrasound can solve all of the above issues. Focused ultrasound achieves a localized mechanical stimulation in a cell non-invasively, and its power for the stimulation can be controlled precisely by its frequency and amplitude. In this study, we originally develop a focused-ultrasound spectroscopic imaging system combined with optical imaging for applying mechanical stimulation on living cell. Optical observation is critically useful in investigating the changes in intracellular organization (e.g., cytoskeletons and nuclear morphology) caused by ultrasound irradiation. No studies, however, appear for observing living cell by ultrasound and optical images at the same time. We successfully monitored living cells for 24 hours by both ultrasound spectrum images and optical images to understand the frequency response of the intercellular ultrasound absorption. Importantly, we found out an ultrasound absorption band at 110~150 MHz, which we attribute to the resonance of the nucleus. This indicates that selective and effective non-invasive mechanical stimulation for the nucleus can be realized by irradiating it with ultrasound of this frequency band.

2. Experimental setup

A low power ultrasound pulse wave with the center frequency of 180 MHz is used to obtain ultrasound images (Fig. 1). It is focused on the culture surface (or lower surface of the cell) by the acoustic lens. The ultrasonic echoes from the upper cell surface and from the culture surface are detected by the same probe, and the latter is used for making the spectroscopic acoustic images (images of varous frequencies) after the Fourier analysis. At the same

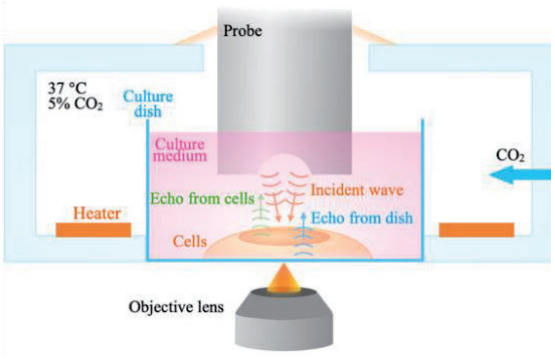

Fig. 1 Experimental setup for obtaining ultrasound spectroscopic images and optical images of cells in culture.

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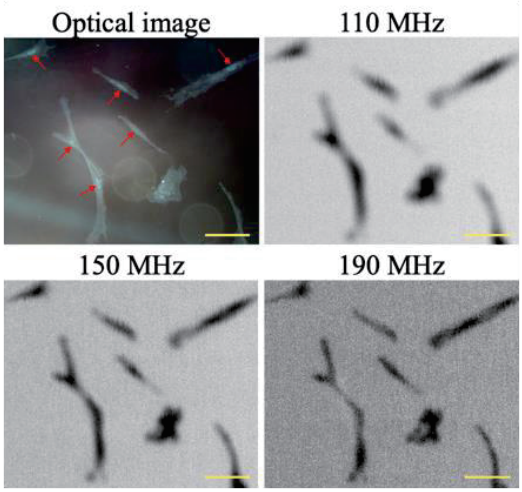

Fig. 2 Optical image and ultrasound absorption images of hMSC at 110 MHz, 150 MHz and 190 MHz. Red arrows in the optical image indicate nuclei. The scale bars indicate 100 μm.

time, the objective lens for optical observation is placed below the culture dish to observe dark-field microscope images from below the transparent culture dish.

In this study, we used human mesenchymal stem cell (hMSC) and human induced pluripotent stem cell (hiPSC) for the observation. The experiment was conducted at 37 ℃ and 5 % CO_2 concentration.

3. Results and discussion

The experimental system we constructed allows us to monitor the living cells for longer than 24 hours. Figure 2 shows the optical image and ultrasound absorption images. The darker area means higher absorption of ultrasonic energy. A higher-frequency image achieves better resolution as expected, but interestingly, images of specific frequencies between 110 and 150 MHz represent nuclei more clearly, indicating that nuclei specifically absorb ultrasonic waves of this frequency band. We calculated the resonance frequencies of an elastic sphere in water with similar elasticity with the cell nucleus and find that its fundamental expansion-contraction resonance mode appears at 154 MHz. Considering that the resonance peak must be significantly broadened and the resonance frequency is lowered because of the viscosity and the nucleus-diameter distribution, the absorption band of 110-150 MHz should be caused by the resonance of the nucleus. This means that ultrasound of this frequency band allows for selective and efficient mechanical stimulation of the nucleus.

We have succeeded in observing the dynamic state of cell division by ultrasound. The cell division

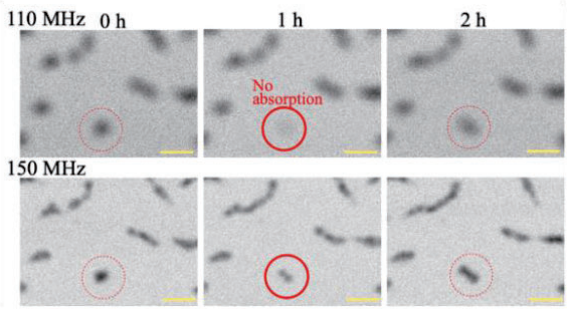

Fig. 3 Ultrasound absorption images of the hiPS cell before and after cell division (marked cell) at 110 and 150 MHz. The scale bars indicate 100 μm.

monitoring supports the resonance of the nucleus: Figure 3 shows the ultrasound absorption images of before and after the cell division. The cell temporarily stopped to absorb ultrasound when it divided because of disassembly of the nuclear membrane. In the optical microscope images, it was difficult to clearly observe the nuclear membrane during the cell division (not shown in this paper), but by acquiring ultrasound absorption images based on the nuclear resonance, the process of nuclear membrane remodeling can be clearly determined.

4. Conclusion

We originally develop a focused ultrasound spectroscopic imaging system combined with optical imaging and monitored living cells for 24 hours or longer. We find out the ultrasound absorption band of 110~150 MHz, which can be attributed to the resonance of the nucleus. This indicates that we can stimulate only the cell nucleus selectively and effectively. Using this original method, we plan to study the effects of ultrasound stimulation to the nuclei in various states on the intracellular morphological changes, differentiation, as well as protein expressions, are closely related to on the cytoskeleton and differentiation.

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