# 1P5-3

# The effect of non-invasive selective mechanical stimulation for the cell nucleus by focused ultrasound

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## 1. Introduction

Living cells are always expose to various mechanical forces such as gravity and blood-flow shear stress, and they have ability to response to them by adjusting their morphology[1] and cell functions[2]. The mechanical stimuli further affect the nuclear-envelope structure, chromatin organization, and gene expression[3-6]. It has been recognized that forces acting on the cell surface and cytoplasm are transmitted to the nuclear envelope through the 'linker of nucleoskeleton and cytoskeleton' (LINC) complex[4]. However, in recent years, more and more evidences appear, indicating that the nucleus detects the mechanical forces independently of the cytoskeleton, and the nuclear-mechanosensing phoenomonon is increasingly attracting attention[3,7]. When understanding its mechanisms, it is difficult to evaluate whether the nucleus detects the chemical signals related to the cytoplasmic mechanotransduction or whether the nucleus itself detects the mechanical forces. Therefore, many of these mechanisms remain unexplained.

One of the serious problems in this field is lack of the technique to apply the mechanical stimulation on only the nucleus noninvasively to separate the contributions of nucleus mechanotransduction from other effects. Previous research applying force on the nucleus surface used micromanipulation[8] or isolation[9]. However, micromanipulation, including pinching[8], cantilever puncture[7], and laser tweezer[10] can cause cell-damaging because of the mechanical contacts and photo-invasiveness due to laser irradiation. Then, the nuclear isolation procedure can change the structure and the chemical composition of the nucleus. They could prevent us from investigating the essence of the nucleus mechanotransduction and monitoring the cell for a long time. Moreover, experiments with isolated nuclei are limited to investigating reactions in the nucleus so that they fail to give the information of the reaction in the cytoplasm induced by the force on the nucleus.

On the other hand, focused ultrasound has a potential to realize a noninvasive localized mechanical stimulation on the living cell. We previously developed an original focused ultrasound system for mechanical stimulation on a cell and propose that irradiating cells with ultrasound of a frequency near 100 MHz can stimulate nucleus selectively due to the nucleus resonance[11]. Here, we apply this system to human mesenchymal stem cells (hMSCs) and investigate the effect of selective stimulation to the nucleus by evaluating laminA/C expression which is known as typical mechanosensory protein[3].

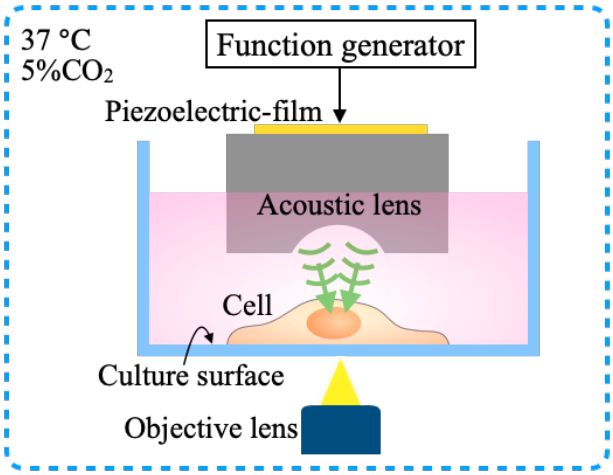


Fig. 1 The developed ultrasound system for the nucleus stimulation in culture environment.

## 2. Experimental setup

The longitudinal plane wave was generated by the piezoelectric-film transducer attached to the top surface of the acoustic probe and focused on the culture surface by the acoustic lens (Fig. 1). Firstly, we obtained ultrasound spectroscopic absorption images of hMSCs at various frequencies to investigate the resonance frequency of the nucleus. A low-power ultrasound pulse wave with a center frequency of 180 MHz was focused to the cell, and the echoes from the culture surface was detected, which was used for constructing the spectroscopic images after the Fourier analysis.

Then, the stimulated position (near the center of the nucleus) was decided by observing the cell with an optical microscope which was placed below the culture dish, and the acoustic probe was moved to the position. The center of the nucleus was irradiated by the burst wave with the resonance frequency for 30 min with the duty cycle of 50%. The experiment was conducted at 37 °C and 5% $CO_2$

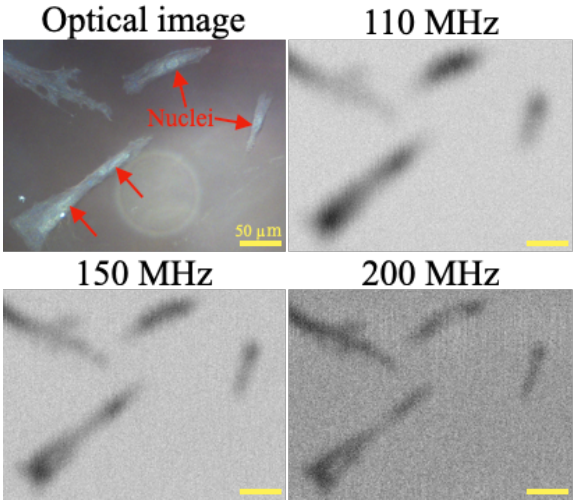


Fig. 2 Optical image and ultrasound absorption images of hMSCs at 110 MHz, 150 MHz and 200 MHz.

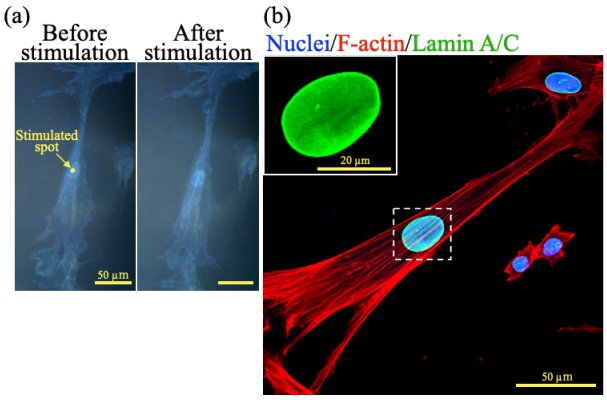


Fig. 3 (a) Optical images of the stimulated cell before and after the stimulation. (b) Immunofluorescence image of the stimulated cell obtained by confocal microscopy which all slice images are stacked. The inset shows the enlarged view of lamin A/C of the white dashed frame which is one slice of the center of the nucleus cross section.

concentration. After the nucleus stimulation, the cells were incubated for 2 hours, and then fixed for immunostaining.

## 3. Results and discussion

Figure 2 shows the optical image and ultrasound absorption images at various frequencies. The darker area indicates higher ultrasonic energy absorption. The results show that nuclear specifically absorb 100-150 MHz ultrasound. This frequency band correspond to the fundamental expansion-contraction resonance frequencies of an elastic sphere with similar elasticity with the cell nucleus, indicating that it is possible to cause resonance of nucleus using this frequency band.

Following this, we conduct the mechanical stimulation by the 110 MHz ultrasound. Figure 3 (a) shows optical images before and after the stimulation. No morphological change was observed during the stimulation, supporting that the stimulation was noninvasive. Then, we evaluate the expression of laminA/C which is protein localized at nuclear membrane (Fig. 3 (b)). The lamin A/C intensity was evaluated by the ratio of the average intensity of nuclear membrane to that of inside the nucleus. In our experiments, the lamin A/C intensity of stimulated cells was about 1.3 times higher than that of unstimulated cells. Our results will play an important role to improve understanding the effect of nucleus mechanical stimulation for gene expression and cell homeostasis.

## 4. Conclusion

We applied originally develop focused ultrasound system for the selective noninvasive mechanical stimulation on the nucleus. We confirmed the nucleus resonance frequency of hMSCs as around 110 MHz and stimulate the nucleus by the 110-MHz frequency burst wave. Then, the expression of lamin A/C was evaluated by immunostaining, which was 1.3 times higher than that of unstimulated cells. We will plan to apply the system for more than 24 hours to study the time and the power dependence of the effect on the lamin A/C expression.

## Acknowledgment

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## References

1) C. Heisenberg, *et al.*, Cell, **153**, 948 (2013).
2) B. Ladoux, *et al.,* Cell, **18**, 743 (2017).
3) T. J. Kirby, *et al.*, Nat. Cell Biol., **20**, 373 (2018).
4) J. Lammerding, *et al.*, APL Bioeng., **6**, 040401 (2022).
5) A. Tajik, *et al.*, Nat. Mater., **15**, 1287 (2016).
6) Y. Kalukula, *et al.*, Mol Cell Biol, **23**, 583 (2022).
7) A. Elosegui-Artola, *et al.*, Cell., **171**, 1397 (2017).
8) G. Fedorchak, *et al.*, Methods Mol. Biol., **1411**, 241 (2014).
9) C. Guilluy, *et al.*, Nat. Cell Biol., **16**, 376 (2014).
10) H. Zhang, *et al.*, J. R. Soc. Interface, **5**, 671 (2008).
11) N. Fujiwara, *et al.*, Jpn. J. Appl. Phys., **63**, 03SP65 (2024).