Orientation control of osteoblast-like cells using ultrasound vibration

超音波振動による骨芽細胞様細胞の配向制御

Serina Tawa[1†], Naoyuki Taya[1], Daisuke Koyama[1]
([1]Doshisha Univ.)
田和　千里奈[1†]，田矢　直之[1]，小山　大介[1]
([1]同志社大学)

1. Introduction

Prevention and noninvasive treatments for bone diseases are required with increase in the number of patients with bone and joint diseases. For bone reconstruction using artificial bone, it is important to control the orientation of anisotropic bone tissue since the fusion and strength at the surface of artificial bones depends on the orientation of bone. Several researchers have reported construction of anisotropic bone tissue and improvement of bone quality by controlling the morphology and arrangement of cells using environment surrounding cells [1]. Orientation of cells can be controlled in parallel or perpendicularly by applying external force such as magnetic fields and shear stress [2],[3] In this study, we focused on a method to control the orientation of osteoblast-like cells using ultrasound vibration. Final goal of our study is the application to artificial bones to enhance the bone fusion. In this paper, we investigated control of the morphology of osteoblast-like cells using ultrasonic vibration and the in vitro experiments were conducted.

2. Experimental methods

2.1 Cell culture

Osteoblast-like cell line (MC3T3-E1) was purchased from the Institute of Physical and Chemical Research, Japan, and used in the in vitro experiments. Osteoblast-like cells are adherent cells derived from a mouse skull and are differentiated into osteoblasts by adding a differentiation-inducing factor (ascorbic acid). The culture medium was prepared by adding fatal bovine serum (Wako Pure Chemical Industries) and penicillin-streptomycin solution (Wako Pure Chemical Industries) to culture solution MEM-a (Wako Pure Chemical Industries). The cells were cultured in an incubator with a CO_2 concentration of 5% and a temperature of 37 °C. The cells were detached from the culture dish using a trypsin solution (Nacalai Tesque) and subcultured twice a week.

2.2 Ultrasound device

Fig. 1 shows the experimental system. An ultrasound vibration device was fabricated to observe the cell growth under ultrasound excitation. The device consists of a culture dish with a glass bottom (diameter: 35 mm; thickness: 0.18 mm) and an annular piezoelectric ultrasound transducer (Fuji Ceramics, C - 213; inner diameter: 10 mm; outer diameter: 20 mm; thickness: 1 mm) polarized in the thickness direction bonded to a circular glass plate (diameter: 27 mm; thickness: 1.1 mm) with an epoxy resin. Considering the acoustic impedance matching between the glass plate and the glass bottom of the dish and disposability of the culture dish, silicone oil was introduced between them as the coupling agents so that the ultrasound vibrations could be generated efficiently on the bottom of the dish. The vibrational distribution was measured by a laser Doppler vibrometer.

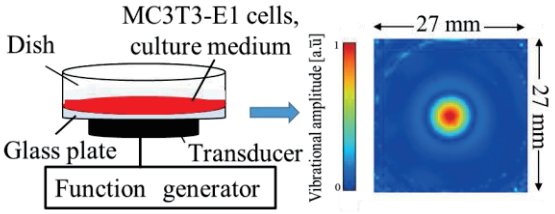


Fig. 1 Experimental setup using an ultrasound vibration device.

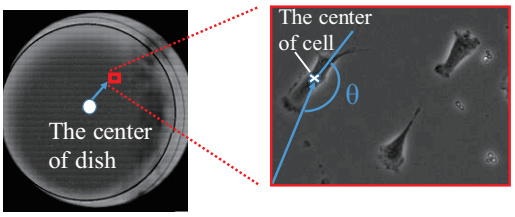


Fig. 2 Microscopic images of the osteoblast-like cells on the culture dish.

2.3 Optical observation under ultrasound excitation

The ultrasound device was installed in a small chamber where the temperature and the CO_2 concentration can be controlled. When a continuous sinusoidal wave at 84 kHz was applied to the ultrasound transducer, the resonance concentric flexural vibration mode was generated on the glass bottom of the dish (see **Fig. 1**). The device was excited for 72 hours with the voltage amplitude of 10 or 17 V. The phase-contrast images were taken with a microscope (IX83, Olympus) before and

after 24, 48, and 72 hours under ultrasound excitation. **Fig. 2** shows the microscopic images of the osteoblast-like cells on the culture dish. The orientation angle of the cells in the cylindrical coordinates θ, which corresponds with the long axis direction of the cell, was measured from the captured images (see **Fig. 2**), and 500 samples were counted in each condition.

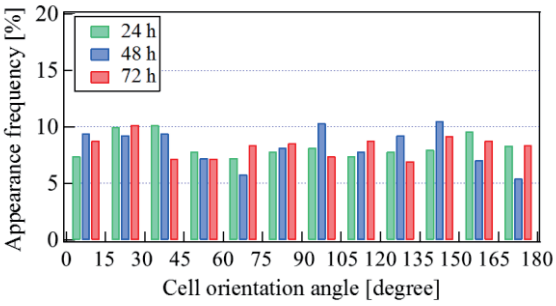
3. Result and discussion

The relationship between the cell growth and the ultrasound vibration was investigated. The histogram of the orientation angle of the cells θ after 24, 48, and 72 hours for the control (0V) and the cases with 10 and 17 V are shown in **Fig. 3**. After 72 hours, the orientation of the cells could not be confirmed. When no input voltage was applied to the ultrasound device (control in **Fig. 3(a)**), the random distribution appeared in the cell orientation. Comparing with the control, the cells were oriented in the circumferential direction (around 90 degrees) of the dish after 24 hours in the case with 10 V (**Fig. 3(b)**). The cells were oriented toward the position with the same ultrasound vibrational amplitude of the glass bottom since the concentric resonance vibration mode was generated on the glass as shown in **Fig. 1**. Larger input voltage to the transducer induced larger affect to the cells by ultrasound vibration as shown in **Fig. 3(c)**. These results implied that the cells were oriented to the position where the vibration amplitude was constant and change in the acoustic radiation force was small spatially since the concentric acoustic field will be generated in the culture medium and the acoustic radiation force can be calculated by the spatial differentiation of sound pressure. **Fig. 4** shows change in the percentage of cells oriented to $\theta = 75$ to 105 degrees when changing the input voltage amplitude from 0 to 17 V. The percentage of the oriented cells in the circumferential direction increased with the voltage amplitude to the ultrasound transducer; they were 16, 22, and 25% for 0, 10, and 17 V, respectively. It should be noted that these percentage decreased with the passage of time. This tendency is attributed to high density of the cells because the surrounding cells affected to the orientation direction of the cells.

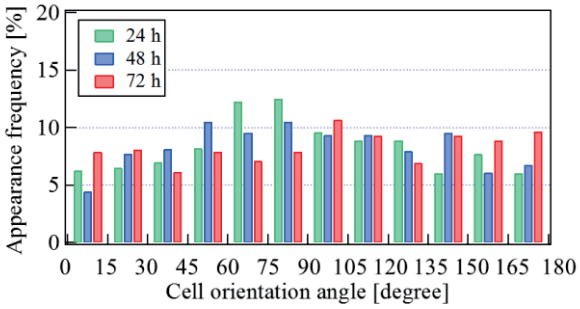
4. Conclusion

A method to control the orientation direction of osteoblast-like cells using ultrasound vibration was discussed. The cells were oriented in the circumferential direction of the culture dish after 24 hours. The effect of orientation control decreased with the passage of time after 48 hours.

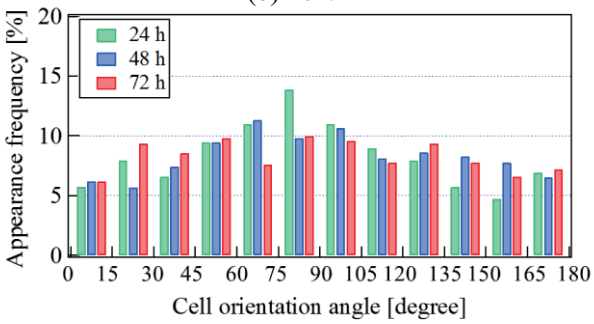
dkoyama@mail.doshisha.ac.jp



(a) Control



(b) 10 V



(c) 17 V

Fig. 3 Histograms of the orientation angle of the cells by ultrasound vibration with several input voltages.

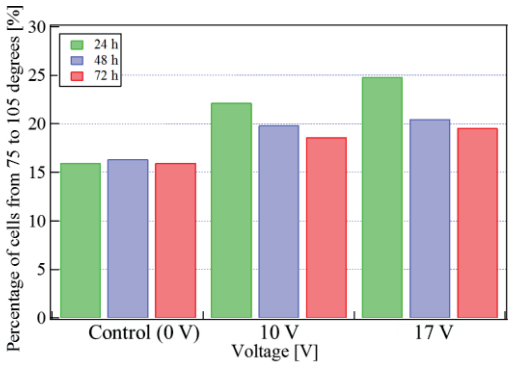


Fig. 4 Change in the percentage of the cells oriented in the circumferential direction (θ = 75 to 105°) with the input voltage.

References

1.K. Ozono, et al: Anti-aging series 3, (2013) p. 293-307
2. H. Kotani, et al.: Journal of the Japan Society of Applied Magnetics, **25** (2001) p. 1167-1170
3. H. Hino, et al.: Transactions of the Japan Society of Mechanical Engineers, **84** (2018) p. 1-11