Detachment of Multiple Adherent Cell Types by Ultrasonic Irradiation to Consumable Cell Culture Dishes

Yuta Kurashina[1,2], Chikahiro Imashiro[2], Kenjiro Takemura[2], [1]Tokyo Institute of Technology, Kanagawa, Japan, [2]Keio University, Kanagawa, Japan

Background, Motivation and Objective

For regenerative medicine and drug discovery research, a large number of cultured cells are required. In mass culture, cells have generally been cultured on culture dishes in a layer. A few days after seeding, cells reach a confluence in the dish and need to be detached from the culture surface. However, it is reported that the protein-degrading enzyme (trypsin), which is generally used to detach cells, lowers the activity of the cells due to damaging the cellular membrane. Therefore, we propose a cell detachment method by using ultrasonic vibration without enzymes. We demonstrate that the method is effective for multiple adherent cell types.

Statement of Contribution/Methods

In the developed device (Fig. 1A), PZT is glued to a glass plate to compose an ultrasonic transducer on which a culture dish locates. Ultrasonic vibration is irradiated to the cells adhered on the culture dish. The input voltage was set to 200 V with a 29 to 31 kHz swept frequency. In addition, the gap between the transducer and the culture dish was filled with glycerol to enhance the transmission of ultrasonic wave.
24 hours after the cell seeding, the cells were immersed in serum-free medium (added the essential nutrients) for 6 hours, and then the cells immersed in FBS were detached by ultrasonic irradiation. The detached cells were counted and reseeded on a culture dish for evaluation of their proliferation. Note that, the cell types we used were CHO (Chinese hamster ovary cell line), C2C12 (Mouse myoblast cell line) and MSC (Human bone marrow-derived mesenchymal stem cells).

Results/Discussion

As shown in Fig. 1B, the same number of cells were able to be detached by using ultrasonic irradiation as the conventional method with trypsin. This result indicates that our method is effective for multiple cell types. In addition, the proliferation of detached cells was improved compared to the conventional method (Data not shown). That is, the activity of detached cells was maintained. From the above, the detachment method by ultrasonic irradiation is superior to the conventional method using enzyme.
As a future work, we should conduct in vivo test with cells obtained by the proposed method for the possible therapies in regenerative medicine.

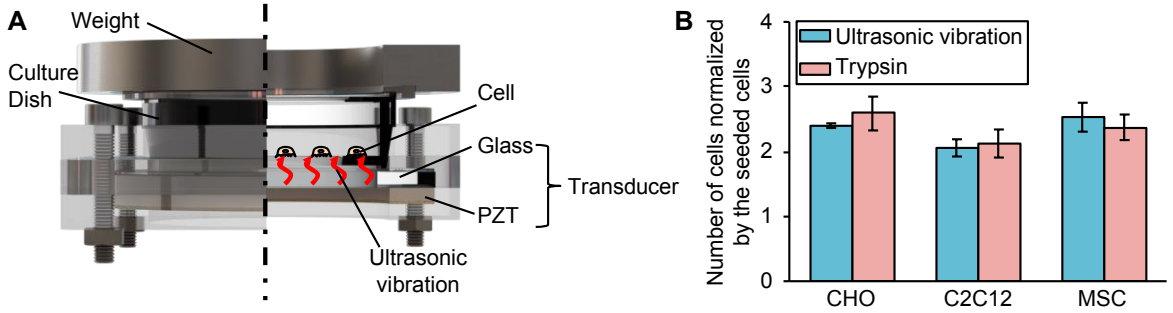

Fig. 1 Cell detachment by using ultrasonic vibration. **A**. Half cross sectional illustration of cell detachment devise composed of transducer, dish and weight. **B**. The cell detachment ratio of CHO, C2C12 and MSC by using ultrasonic vibration (mean ± SD, $n = 4$). The number of cells were normalized by the seeded cells.