World Academy of Science, Engineering and Technology
International Journal of Electrical and Information Engineering
Vol:11, No:06, 2017

Acoustic Radiation Pressure Detaches Myoblast from Culture Substrate by Assistance of Serum-Free Medium

Authors : Yuta Kurashina, Chikahiro Imashiro, Kiyoshi Ohnuma, Kenjiro Takemura

Abstract : Research objectives and goals: To realize clinical applications of regenerative medicine, a mass cell culture is highly required. In a conventional cell culture, trypsinization was employed for cell detachment. However, trypsinization causes proliferation decrease due to injury of cell membrane. In order to detach cells using an enzyme-free method, therefore, this study proposes a novel cell detachment method capable of detaching adherent cells using acoustic radiation pressure exposed to the dish by the assistance of serum-free medium with ITS liquid medium supplement. Methods used In order to generate acoustic radiation pressure, a piezoelectric ceramic plate was glued on a glass plate to configure an ultrasonic transducer. The glass plate and a chamber wall compose a chamber in which a culture dish is placed in glycerol. Glycerol transmits acoustic radiation pressure to adhered cells on the culture dish. To excite a resonance vibration of transducer, AC signal with 29-31 kHz (swept) and 150, 300, and 450 V was input to the transducer for 5 min. As a pretreatment to reduce cell adhesivity, serum-free medium with ITS liquid medium supplement was spread to the culture dish before exposed to acoustic radiation pressure. To evaluate the proposed cell detachment method, C2C12 myoblast cells (8.0 × 104 cells) were cultured on a ø35 culture dish for 48 hr, and then the medium was replaced with the serum-free medium with ITS liquid medium supplement for 24 hr. We replaced the medium with phosphate buffered saline and incubated cells for 10 min. After that, cells were exposed to the acoustic radiation pressure for 5 min. We also collected cells by using trypsinization as control. Cells collected by the proposed method and trypsinization were respectively reseeded in ø60 culture dishes and cultured for 24 hr. Then, the number of proliferated cells was counted. Results achieved: By a phase contrast microscope imaging, shrink of lamellipodia was observed before exposed to acoustic radiation pressure, and no cells remained on the culture dish after the exposed of acoustic radiation pressure. This result suggests that serum-free medium with ITS liquid inhibits adhesivity of cells and acoustic radiation pressure detaches cells from the dish. Moreover, the number of proliferated cells 24 hr after collected by the proposed method with 150 and 300 V is the same or more than that by trypsinization, i.e., cells were proliferated 15% higher with the proposed method using acoustic radiation pressure than with the traditional cell collecting method of trypsinization. These results proved that cells were able to be collected by using the appropriate exposure of acoustic radiation pressure. Conclusions: This study proposed a cell detachment method using acoustic radiation pressure by the assistance of serum-free medium. The proposed method provides an enzyme-free cell detachment method so that it may be used in future clinical applications instead of trypsinization.

Keywords : acoustic radiation pressure, cell detachment, enzyme free, ultrasonic transducer
Conference Title : ICU 2017 : International Conference on Ultrasonics
Conference Location : Paris, France
Conference Dates : June 25-26, 2017