

THE DEVELOPMENT OF SCALABLE BIOREACTOR SERIES FOR HUMAN INDUCED PLURIPOTENT STEM CELL STIRRED SUSPENSION CULTURE

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Introduction

Induced pluripotent stem (iPS) cells are promising cell sources for regenerative medicine. The stirred suspension culture using a bioreactor system is an efficient method for the large scale expansion of human iPS cells. On the basis of our hypothesis that a low shear stress and the uniform medium flow in a vessel are important for the suspension culture of human iPS cells, we showed the bioreactor system which is capable of the stirred suspension culture of the inoculated single iPS cells in the aggregates condition at the last this conference. We designed the bioreactor which expanded culture volume to 5L, and tried scale up culture from 100mL this time.

Material and method

At first we designed the stirrer including delta shape paddle impeller for 100mL, 500mL, 5L bioreactor. These impellers similar design prevent a turbulent flow and agitate medium by laminar flow. The DO sensor and pH electrode were used for maintain the culture condition. The single cell suspension was prepared from 2D feeder free culture and inoculated into 100mL bioreactor as passage 1 (1 to 2×10^5 cells/mL). The cell aggregates were collected after the stirred suspension culture for 4 days, and dissociated into the single cells by using trypsin. The culture period was adjusted by proliferation of cell. The obtained single cells were re-inoculated into 500mL bioreactor and stirred suspension culture was performed as passage 2. After the culture of passage 2, obtained single cell suspension re-inoculated into 5L bioreactor as passage 3. The number of viable cells, and the number and size of aggregates were measured. The undifferentiated property of the expanded iPS cells was evaluated with the flow cytometric analysis.

Results and discussion

The large scale culture using the delta shape paddle impeller enabled to create a lot of aggregates 200 to 300 micrometer diameter in the cultivation for 4 days. Furthermore, the same cell proliferation was observed in these three bioreactors and the number of cells increased 10 folds for 4 days. Finally, 6.7×10^9 cells were proliferated in 5L bioreactor. The flow cytometric analysis revealed that more than 90% of population maintained undifferentiated ability in all the passages. Collectively, we established the methods for the expansion of human iPS cells to 10 billion scale with undifferentiated state.