HIGH DENSITY CULTURE OF HUMAN INDUCED PLURIPOTENT STEM CELLS THROUGH THE REFINEMENT OF MEDIUM BY DIALYSIS IN SUSPENSION

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Human induced pluripotent stem cells (hiPSCs) hold great promise in the field of regenerative medicine for cell-based therapies, tissue engineering, and drug discovery because of their pluripotency and self-renewal capacity. To implement their potential, bio-process developments for robust expansion of hiPSCs are important since large numbers of hiPSCs are required for cell therapy application. Although suspension culture is superior to obtain large numbers of cells, the cost of culturing hiPSC increases with increasing medium consumption, as the culture medium contains many costly macromolecules including basic fibroblast growth factor (bFGF), transforming growth factor beta 1 (TGF-β1), and insulin. Moreover, hiPSCs secrete essential autocrine factors that are removed along with toxic metabolites when the growth medium is exchanged daily. In this study, after determining the minimum inhibitory level of lactic acid for hiPSCs, a medium refining system was constructed by which toxic metabolites were removed from used culture medium and autocrine factors as well as other growth factors were recycled. Specifically, about 87% of bFGF and 80% of TGF-β1 were retained in the refined medium after dialysis. The refined medium efficiently potentiated the proliferation of hiPS cells in adherent culture. When the refining system was used to refresh medium in suspension culture, a final cell density of $(1.1 \pm 0.1) \times 10^6$ cells mL^{-1} was obtained, with 99.5 ± 0.2% OCT 3/4 and 78.3 ± 1.1% TRA-1-60 expression, on day 4 of culture. These levels of expression were similar to those observed in conventional suspension culture. Moreover, to obtain high density culture, size- and time-dependent boundary conditions were also considered for the preferable growth of hiPSC in suspension culture. Thus, the concept for high density culture was proposed by considering the boundary conditions for preferable growth of hiPSC, as well as, medium refinement by dialysis to remove toxic metabolites, recycle autocrine factors, and reduce the use of macromolecules for the reduction of culture cost in suspension.