EXPANSION OF 3D HUMAN INDUCED PLURIPOTENT STEM CELL AGGREGATES IN BIOREACTORS: BIOPROCESS INTENSIFICATION AND SCALING-UP APPROACHES

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Human induced pluripotent stem cells (hiPSC) are attractive tools for drug screening and disease modeling and promising candidates for cell therapy applications. However, to achieve the high numbers of cells required for these purposes, scalable and clinical-grade technologies must be established.
In this study, we use environmentally controlled stirred-tank bioreactors operating in perfusion as a powerful tool for bioprocess intensification of hiPSC production. We demonstrate the importance of controlling the dissolved oxygen concentration at low levels (4% oxygen) and perfusion at 1.3 day^{-1} dilution rate to improve hiPSC growth as 3D aggregates in xeno-free medium (Cellartis® DEF-CS™ 500 Xeno-Free Culture Medium). This strategy allowed for increased cell specific growth rate, maximum volumetric cell concentrations ($4.7x10^6$ cell/mL) and expansion factors (approximately 19), resulting in an overall improvement of 2.6-fold in cell yields. Extensive cell characterization, including whole proteomic analysis was performed to confirm that the pluripotent phenotype was maintained during culture.
Furthermore, a scalable protocol for continuous expansion of hiPSC aggregates in bioreactors was implemented using mechanical dissociation protocols for aggregate disruption and cell passaging. A total expansion factor of 1100 in viable cells was obtained in 11 days of culture (Figure 1), while cells maintained their proliferation capacity, pluripotent phenotype and potential as well as genomic stability after 3 sequential passages in bioreactors. To our knowledge, this is the highest expansion factor reported for hiPSC for such a short culture time frame. The strategy described herein for continuous expansion of hiPSC provides important insights towards up-scale production of hiPSC. This will strengthen the utility of hiPSC in cell therapy, drug discovery, toxicity testing and disease modeling.

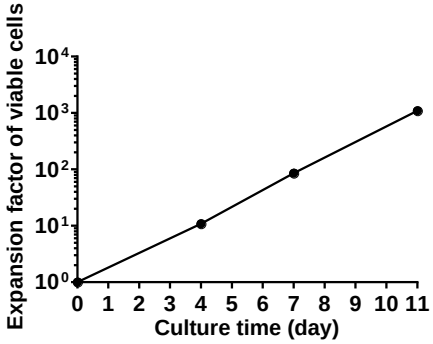

Figure 1 – Cumulative expansion factor of viable hiPSC obtained through three sequential passages in stirred-tank bioreactors.

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