BIOPROCESS INTENSIFICATION FOR THE CONTINUOUS EXPANSION OF 3D HUMAN INDUCED PLURIPOTENT STEM CELL AGGREGATES IN BIOREACTORS

Bernardo Abecasis, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
babecasis@itqb.unl.pt
Tiago Aguiar, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Émilie Arnault, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Rita Costa, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Patricia Gomes-Alves, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Anders Aspegren, Takara Bio Europe AB, Gothenburg, Sweden
Margarida Serra, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal
Paula M. Alves, iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

Key Words: Human induced pluripotent stem cells, Stirred tank bioreactors, Aggregate dissociation, Continuous Bioprocess, Scale-up

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. Firstly, 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. Secondly, we have tested different chemical and mechanical strategies for hiPSC aggregate dissociation, revealing similar viable cell recovery yields (approximately 50%). However, only the mechanical dissociation strategies enabled the re-aggregation of hiPSC in stirred conditions, with the mechanical dissociation using a 70 µm pore size nylon mesh allowing a higher expansion factor after dissociation. Finally, a scalable protocol for continuous expansion of hiPSC aggregates in bioreactors was implemented using the mechanical dissociation for aggregate disruption/passaging. A total expansion factor of 1100 in viable cells was obtained in 11 days of culture after 3 sequential passages in bioreactors, while cells maintained their proliferation capacity, pluripotent phenotype and potential as well as genomic stability. 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-scaling the production of hiPSC. Integrative biomanufacturing processes using this continuous strategy are now being pursued for hiPSC expansion and differentiation towards cardiac lineages in order to recreate cardiac models for drug discovery, toxicity testing and disease modeling.

Acknowledgments: This work was supported by Fundação para a Ciência e Tecnologia (FCT)-funded projects CARDIOSTEM (MITP-TB/ECE/0013/2013) and CardioRegen (HMSP-ICT/0039/2013); and iNOVA4Health UID/Multi/04462/2013, a program supported by FCT/Ministério da Educação e Ciência, through national funds and cofounded by FEDER under the PT2020 Partnership Agreement. BA. was supported by FCT Grant SFRH/BD/52475/2013. The work was also funded by a Vinnova Grant, registration number 2014-00310 (Cell therapies via large scale expansion of pluripotent stem cells).