HUMAN PLURIPOTENT STEM CELL EXPANSION IN VERTICAL-WHEEL BIOREACTORS

Carlos A.V. Rodrigues, iBB-Institute for Bioengineering and Biosciences and The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
carlos.rodrigues@tecnico.ulisboa.pt
Diogo E.S. Nogueira, iBB-Institute for Bioengineering and Biosciences and The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Cláudia C. Miranda, iBB-Institute for Bioengineering and Biosciences and The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal
Yas Hashimura, PBS Biotech, CA, USA
Sunghoon Jung, PBS Biotech, CA, USA
Brian Lee, PBS Biotech, CA, USA
Joaquim M.S. Cabral, iBB-Institute for Bioengineering and Biosciences and The Discoveries Centre for Regenerative and Precision Medicine, Lisbon Campus, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal

Key Words: human induced pluripotent stem cells; Vertical-Wheel Bioreactors; microcarriers; aggregates.

Human induced pluripotent stem cells (hiPSC) have been regarded as an enormous breakthrough for medicine, since they can be derived from patients and be used to generate virtually all types of cells in the human body. One of the great bottlenecks in the usage of these cells for regenerative medicine or drug discovery applications is their expansion to relevant quantities. The Vertical-Wheel Bioreactors (PBS Biotech) present a novel scalable bioreactor configuration, whose agitation mechanism allows for homogeneous mixing conditions inside the single-use vessel, while conveying less shear stress to the cells when compared to traditional alternatives. These characteristics are advantageous for hiPSC expansion and thus, in this work, hiPSC were expanded in the Vertical-Wheel Bioreactor using different strategies, namely culturing the cells 1) on microcarriers and 2) as floating aggregates.

In the first approach, cells were cultured under xeno-free conditions, using the Essential 8 medium together with microcarriers and coatings devoid of any animal-derived products [1]. The culture conditions were optimized in terms of initial cell/microcarrier ratio, inoculation method and agitation rate, in the PBS 0.1 vessel (working volume: 80 mL). The cells were successfully expanded, maintaining a normal karyotype, up to a 6.7-fold increase in cell number, after 6 days. These optimized culture conditions were successfully repeated in a larger vessel, the PBS 0.5 (300 mL working volume) demonstrating the scalability of the Vertical-Wheel system.
In the second approach, hiPSC were expanded as floating aggregates, a methodology which does not require a separation step at the end of culture, to remove microcarriers, facilitating the downstream processing and Good Manufacturing Practice-compliance of the process. Cells were cultured in the PBS 0.1 (working volume: 60 mL), using mTeSR1, a serum-free medium and were monitored throughout culture regarding growth kinetics, aggregate size distribution and expression of pluripotency markers. The Vertical-Wheel Bioreactors were shown to efficiently keep the cell aggregates in suspension, under lower linear agitation speeds than an equivalent volume spinner flask (7 cm/s vs. 13 cm/s). Following 7 days of culture, cells were expanded up to a 5.2 ± 0.6-fold increase in cell number. The hiPSC aggregates increased in size over time, from an average diameter of 135 ± 61 µm to 397 ± 119 µm after 7 days. Pluripotency was maintained throughout time, as assessed by sustained high (> 80%) expression of pluripotency markers OCT4, SOX2 and TRA-1-60, and low (< 10%) expression of early differentiation marker SSEA-1. The results were validated using a second hiPSC line.
This study revealed that the Vertical-Wheel Bioreactor allows hiPSC growth either on microcarriers and as aggregates and suggested it to have advantages versus other configurations. These results make the Vertical-Wheel Bioreactor a promising platform for hiPSC expansion and, prospectively, differentiation approaches, contributing for the generation of *bona fide* cells for various biomedical applications, namely drug screening, disease modelling, and, ultimately, for Regenerative Medicine.

[1] Rodrigues CAV, Silva TP, Nogueira DES, Fernandes TG, Hashimura Y, Wesselschmidt R, Diogo MM, Lee B, Cabral JMS (2018), “Scalable Culture Of Human Induced Pluripotent Cells On Microcarriers Under Xeno-Free Conditions Using Single-Use Vertical-Wheel™ Bioreactors”, Journal of Chemical Technology and Biotechnology, DOI: 10.1002/jctb.5738