DEVELOPMENT OF A SCALE-DOWN APPROACH TO THE SCALABLE CULTURE OF INDUCED PLURIPOTENT STEM CELLS ON MICROCARRIERS USING SINGLE-USE VERTICAL-WHEEL™ BIOREACTORS UNDER XENO-FREE CONDITIONS

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Induced Pluripotent Stem Cells (iPSC) are capable of extensive self-renewal while retaining the ability to differentiate into virtually all cell types of the body. These cells are the subject of much research and development activity aimed at the development of cell-based tools, which may speed drug discovery, and cell-based medical therapies that are being developed to address unmet medical needs. However, development of these therapies is hampered by manufacturing bottlenecks including production scale up to meet the anticipated demand. PBS Biotech, Inc. has developed a single use bioreactor with an innovative Vertical-Wheel™ design that promotes more homogenous and gentle particle suspension, under lower hydrodynamic shear environment than traditional bioreactor vessel design. Vertical-Wheel bioreactors are available from lab-scale vessels (PBS MINI) to larger production units (up to 500L). This study describes the culture of human iPSCs on microcarriers under xeno-free conditions using Vertical-Wheel bioreactors. Human iPSCs were cultured on microcarriers to provide surface for cell attachment using the chemically defined Essential 8 culture medium, a xeno-free, feeder-free culture medium. The culture conditions were optimized in terms of 1) initial cell/microcarrier ratio, 2) inoculation method and 3) agitation rate, in the PBS-0.1 vessel using 80 mL working volume. The cells were successfully expanded, up to a 7-fold increase in cell number, after 6 days in the bioreactor. Glucose consumption and lactate production were analyzed to prevent glucose starvation or excessive lactate accumulation. These optimized culture conditions were successfully repeated in a larger vessel, the PBS-0.5 using 300 mL working volume, demonstrating the scalability of the Vertical-Wheel system. With this PBS-0.5 bioreactor, 3 x 10^8 cells were produced after 6 days of operation, and the specific growth rate (0.72 day^{-1}) was similar to the one observed with the PBS-0.1 (0.68 day^{-1}). The applications of iPSC cells and their progeny, especially in clinical settings, will require a guarantee of cell quality. After PBS-MINI bioreactor culture, the expression of pluripotency markers, such as Oct4, Nanog, and SSEA4 was assessed by immunocytochemistry and flow cytometry. The directed differentiation into the neural lineage of the expanded cells was performed and the pluripotency of the cells was further tested after embryoid body formation. The robustness of this process method was evaluated by cultivating another iPSC cell line under the same process conditions, resulting in identical growth kinetics in the PBS MINI-0.1. The methodology developed herein, which grows human iPSC on microcarriers in single-use bioreactors using chemically defined xeno-free cultivation reagents provides a foundation upon which further refinement and scale-up of processes can be built for large scale production of iPSCs.