APPLICATION NOTE | PBS-0.1 MINI S.U.B.

The use of Vertical-Wheel® technology for superior culture homogeneity and expansion of hiPSCs

INTRODUCTION

Due to their high in-vitro proliferation capacity and ability to differentiate into all three germ layers of the human body, human induced pluripotent stem cells (hiPSCs) hold extraordinary potential to accelerate breakthroughs in cell and gene therapy, drug screening, and disease modeling. However, the lack of standardized protocols and challenges with large-scale expansion required to meet the large quantity of high-quality cells required for clinical dosage have prevented many advances in the field. To demonstrate how the combination of optimized protocols with Vertical-Wheel® technology can overcome hiPSC workflow bottlenecks, a 6-day expansion was performed comparing the PBS-0.1 Vertical-Wheel® Bioreactor to the traditionally used horizontal-blade bioreactor. Aggregate sizing and size distribution was determined, and fold-expansion was calculated.

MATERIALS & METHODS

Single-cell inoculation

hiPSCs were expanded in static culture before being dissociated into single cells for inoculation. Cells were counted before being added directly into the agitated bioreactor culture. hiPSCs were inoculated into 100-mL working volume horizontal-blade glass bioreactors (Corning Style Spinner Flask, NDS Technologies Inc.) and 100 mL working volume single-use PBS-0.1 Mini (PBS Biotech, Inc.) at a density of 20,000 cells/mL.

Expansion

hiPSCs were cultured at maximum working volume (100 mL) of mTeSR1 medium supplemented with 10 µM Y-27632. Constant mixing was maintained at agitation rates of 40, 60, and 80 rpm in standard culture conditions of 37°C and air with 5% CO_2 on overlay with no medium exchange (batch) for a culture period of 6 days.

Cell counts

For this study, cell samples were collected (n=4) from bioreactors for each condition and cell counts were performed in duplicates.Samples were removed during agitation using serological pipettes to minimize settling of aggregates. Samples were centrifuged at 300 g for 5 minutes.The supernatant was discarded, and cell pellet resuspended in 1.0 mL of Accutase and left in 37°C water bath for 5-7 minutes. The cell solution was gently pipetted 3 times before 1 mL of medium was added to dilute the enzyme. The sample was centrifuged at 500 g for 5 minutes, supernatant discarded, and pellet resuspended in 0.5-1.0 mL medium. Two, 200 µL aliquots were taken from each cell sample for viable cell counts using the NucleoCounter (NC-200) (ChemoMetec, Denmark).

Aggregate sizing and size distribution

To determine average aggregate size and size distributions, 1.5-mL samples were removed using a serological pipette from the bioreactors and added into 12-well plates for visualization. Images were taken using a Zeiss Axiovert 25 microscope (Carl Zeiss) with AxioVision software for measurements. Aggregates were defined as multi-cellular spheroids with a diameter greater than 50 µm. Diameter was determined by taking the average of the greatest length across the aggregate and the length perpendicular to the greatest length. A minimum of 100 aggregates were sized per condition.

RESULTS

Cell Growth

Single-cell inoculation of hiPSCs in the Vertical-Wheel without media exchanges was quite successful, reaching a maximum expansion of 16.7 ± 1.1-fold at 40 rpm (Figure 1). In contrast, fold expansion in the horizontal-blade bioreactor was minimal, reaching a maximum of 6.3 ± 2.7-fold at 80 rpm, likely due to poor mixing that results in large, heterogeneous aggregates (Figure 2).

The use of Vertical-Wheel® technology for superior culture homogeneity and expansion of hiPSCs

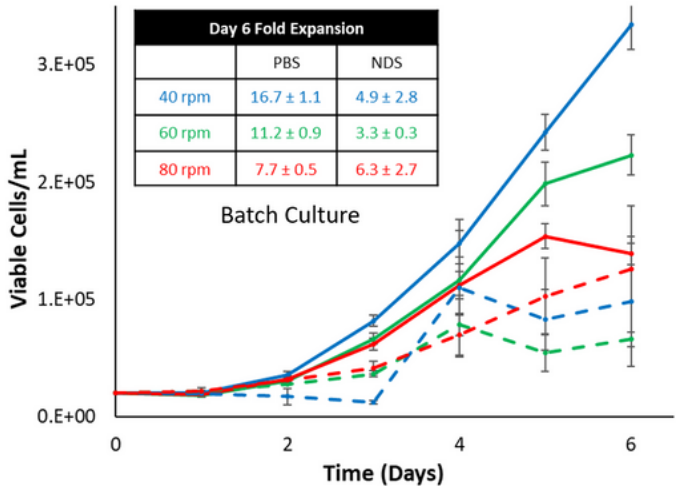

Figure 1. hiPSCs cultured in the PBS-0.1 Mini Vertical-Wheel Bioreactor exhibit superior expansion in comparison to hiPSCs cultured in a horizontal-blade bioreactor at 40, 60, and 80rpm.

Aggregate size and distribution

The graphed aggregate distribution shows bi-modal peaks or large, flat distributed averages in the horizontal-blade bioreactor, indicative of unhealthy aggregate morphology, and have a diameter over 400µm by day 5 (Figure 3). Conversely, hiPSCs cultured in the Vertical-Wheel bioreactors maintained consistent aggregate size with a single, narrow peak distribution at all tested agitation rates.Day 5 average aggregate size in the Vertical-Wheel bioreactor remained below the threshold aggregate size, ranging between 169 µm ± 5.5 µm and 275 µm ± 6.9 µm in diameter at 80 rpm and 40 rpm, respectively.

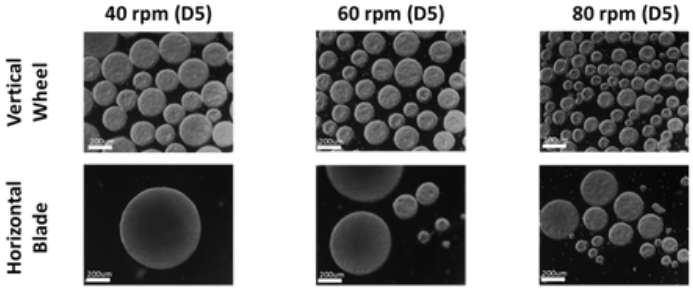

Figure 2. Single-cell inoculated hiPSCs cultured with no media exchanges in horizontal-blade bioreactor exhibit large aggregates at each agitation rate. Scale bar = 200 µm. Representative of brightfield microscopic images.

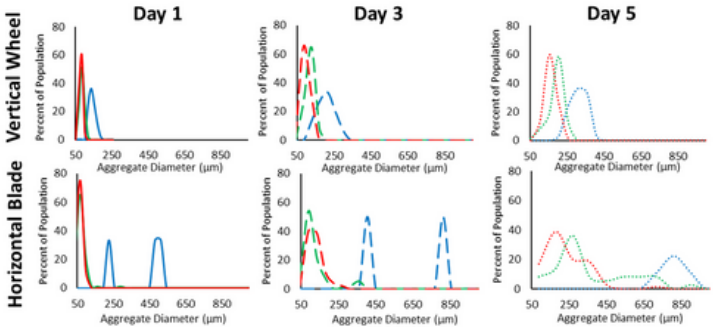

Figure 3. Horizotal-blade bioreactors exhibit heterogeneous aggregate sizes illustrated here by bi-modal peaks or large, flat distributed averages. Vertical-Wheel bioreactors demonstrate a consistent aggregate size at various agitation rates.

DISCUSSION

While horizontal-impeller bioreactors are traditionally used for 3D culture, the complex hydrodynamics and shear-sensitivity of iPSCs requires alternative technology to overcome culture heterogeneity and limited expansion which hinders the advancement of iPSCs for use in allogeneic cell and gene therapy. Additionally, horizontal-blade or turbine impellers require careful optimization at each scale, limiting success with iPSCs due to the increasing shear stress at the impeller tip which also increases with reactor scale. The Vertical-Wheel technology utilized for this study is unique in its mixing ability which results in uniform distribution of hydrodynamic forces and a lower shear stress environment, ideal for hiPSC growth as aggregates. Additionally, the hydrodynamic distribution of energy dissipation rate scales exponentially, controlling aggregate size and size distribution when scaling into larger Vertical-Wheel bioreactors, greatly reducing scale-up optimization time. PBS Biotech offers clinical-grade Vertical-Wheel bioreactors in several sizes to accommodate research through commercial scale applications, making it a solid choice for clinical iPSC process development.

REFERENCES

Borys BS, Dang T, So T, Rohani L, Revay T, Walsh T, Thompson M, Argiropoulos B, Rancourt DE, Jung S, Hashimura Y, Lee B, Kallos MS. Overcoming bioprocess bottlenecks in the large-scale expansion of high-quality hiPSC aggregates in vertical-wheel stirred suspension bioreactors. Stem Cell Res Ther. 2021 Jan 13;12(1):55. doi: 10.1186/s13287-020-02109-4. PMID: 33436078; PMCID: PMC7805206.

ORDERING INFORMATION

Product	Part Number
PBS Mini Bioreactor Base Unit	FA-UNI-B-501
PBS-0.1 Mini Single-Use Vessels (4-pack)	FA-0.1-D-001
PBS-0.5 Mini Single-Use Vessels (4-pack)	FA-0.5-D-001
PBS-3 Vertical-Wheel Bioreactor	IA-3-B-701
PBS-3 Single-Use Vessel, SUS	FA-3-D-706-L
PBS-15 Vertical-Wheel Bioreactor	IA-15-B-501
PBS-15 Single-Use Vessel	IA-15-D-506-L
PBS-80 Vertical-Wheel Bioreactor	IA-80-B-511
PBS-80 Single-Use Vessel	IA-80-D-511-L

For more information, please contact your account manager at **sales@pbsbiotech.com**.

To place an order, please contact customer service at **customer.service@pbsbiotech.com**.

PBS BIOTECH®
www.pbsbiotech.com
4721 Calle Carga, Camarillo, CA 93012
Phone +1 805 482-7272