APPLICATION NOTE | PBS-0.1 MINI & PBS-3 S.U.B.

Linear scale-up of hiPSC cell lines using Vertical-Wheel® Bioreactors

INTRODUCTION

The use of human induced pluripotent stem cells (hiPSCs) for cell and gene therapy development is gaining in popularity due to their capacities for self-renewal and differentiation into a multitude of cell types, without the ethical sourcing concerns and immune rejection associated with traditional embryonic stem cells. Production of hiPSCs using vessels with horizontal impellers can lead to varying results in cell yield and quality especially during scale up, which ultimately elongates the time and cost of manufacturing development efforts. This study demonstrates how Vertical-Wheel® Bioreactors maintain optimal and consistent cell yield, morphology, and surface marker expression when scaling-up from PSC aggregate culture from 100mL to 3L working volumes.

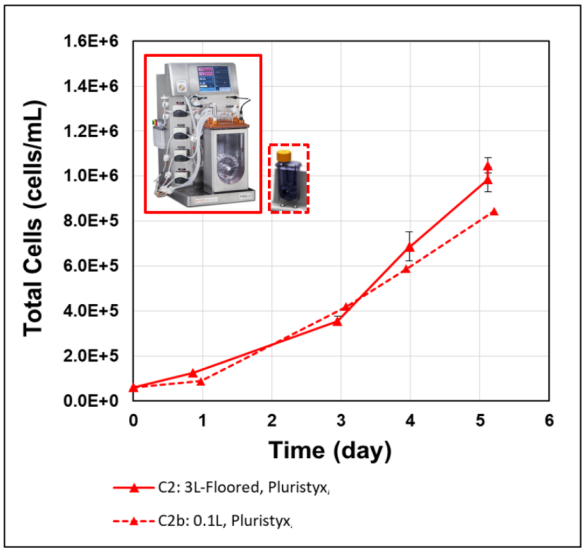
MATERIALS & METHODS

One vial of TC1133 hiPSCs was thawed and seeded into two T-75 flasks containing mTESR™1 medium (Stem Cell Technologies) supplemented with 10µM Y-272632 at a density of 15,000 cells/cm². These cells were cultured for 72 hrs then passaged into six T-175 flasks at a density of 5,000 cells/cm² to be cultured for 90hrs. Cells were inoculated into 0.1L and 3L Vertical-Wheel Bioreactors at 50,000 cells/mL and expanded in TeSR™-AOF 3D medium (STEMCELL™ Technologies) for scale-up comparison. The corresponding 0.1L and 3L bioreactor agitation rates of 60rpm and 24rpm respectively were scaled using a constant power/volume correlation. The feeding strategy in the large-scale reactor included a bolus addition on day 1 and day 2 followed by perfusion feeding at a rate of 0.5 VVD from day 3 and day 5. This allowed for uninterrupted agitation throughout the culture period. pH was monitored daily during medium exchanges and found to remain above 6.8 throughout the culture period. This same workflow was utilized for the PLX1 (Pluristyx) hiPSC line for comparison.

RESULTS

Expansion

Under these operating conditions, both cell lines demonstrated predictable growth and linear scale-up, maintaining cells/mL concentrations and nearly identical overall specific growth rates between the 0.1L and 3L bioreactor systems. No notable cell loss or lag phase after inoculation (~1 cell population doubling between day 0 and day 1) (Figure 1). Glucose and lactate profiles (not shown) were consistent across scales over the entirety of the cell culture, which supports cell count accuracy and consistency between conditions.



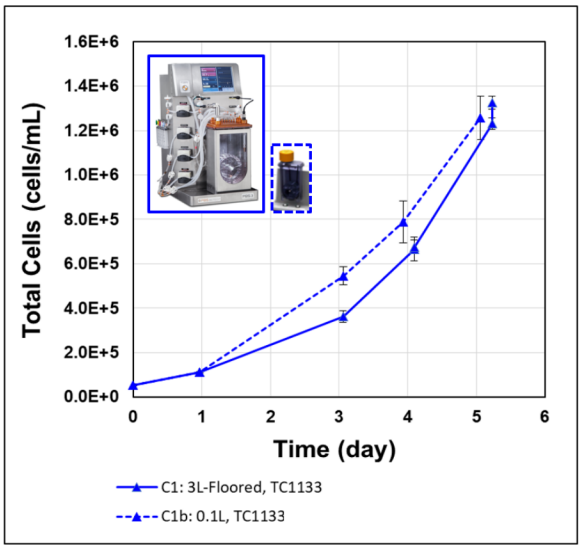


Figure 1. TC1133 and PLX1 cell lines demonstrate consistent growth and linear production scale-up. Dashed lines are representative of samples taken from PBS-0.1 vs. solid lines which are taken from the PBS-3. No significant cell loss or lag phase experienced post-inoculation.

Morphology

There is evident aggregate formation and few single cells on day 1, followed by healthy and consistent growth of aggregates throughout the 5 days of culture for both cell lines and bioreactor scales (Figure 2).

Surface marker expression

Day 5 aggregate samples from the PBS-3 Vertical-Wheel Bioreactors were dissociated into single cells for phenotypic assessment. High levels of pluripotency surface markers SSEA-4 and TRA-1-60, and nuclear markers SOX-2 and OCT-4 were demonstrated for both TC1133 and PLX1 cell lines (Figure 3).

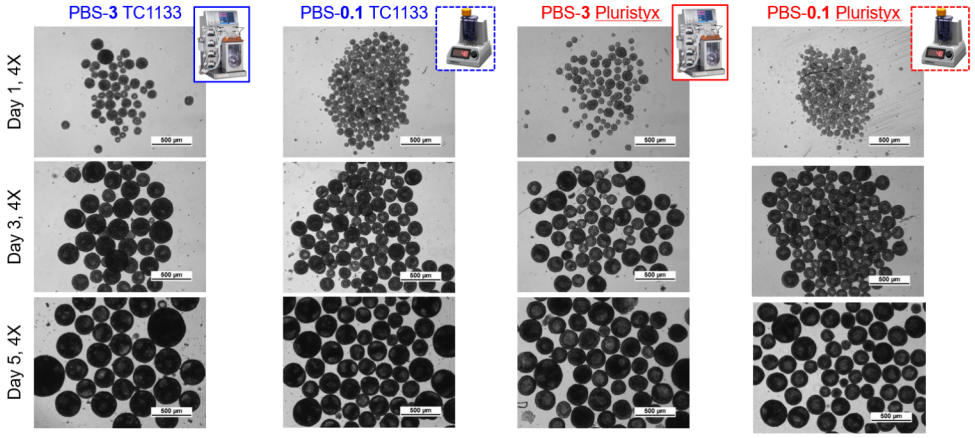


Figure 2. Phase contrast microscope images are representative samples from the 0.1L and 3L bioreactor on day 1, day 3, and day 5. Aggregate formation at day 1, followed by aggregate growth and morphology through day 5 is demonstrated to be healthy and consistent for both cell lines and bioreactor scales.

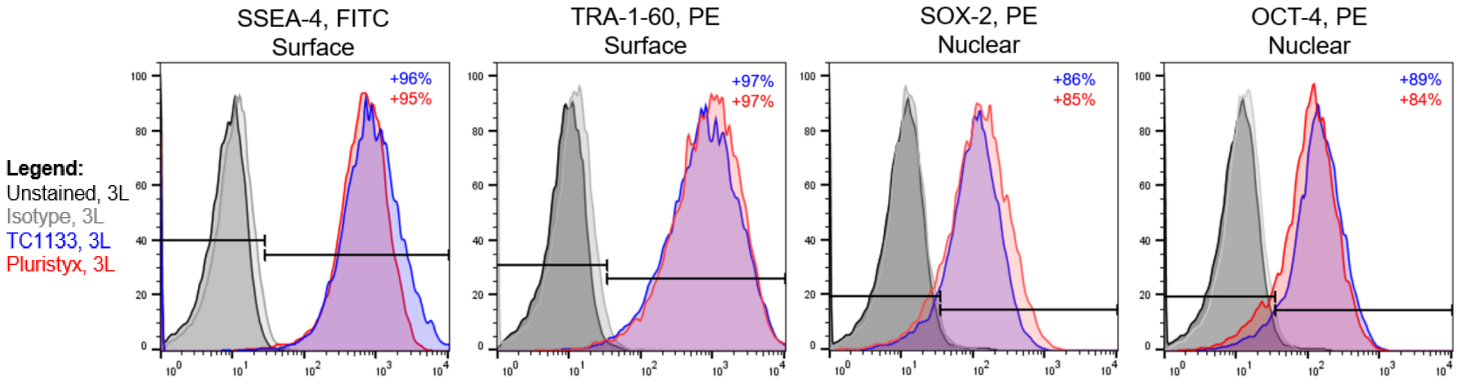


Figure 3. TC1133 and PLX1 cell lines maintained high levels of pluripotency marker expression on day 5 of expansion in the PBS-3 bioreactor systems.

DISCUSSION

Healthy hiPSC aggregate morphology and homogeneity is important for hiPSC expansion and downstream applications as aggregate size control will increase the overall quality of the culture and can impact directed differentiation efficiency. By establishing a narrow distribution of aggregate sizes, the maximum yield potential is increased because large aggregates encounter oxygen and nutrient diffusion limitations within themselves. Small-scale Vertical-Wheel Mini vessels are valuable as a tool for parallel testing and ranging or factorial studies during process development and optimization. They only hold this value, however, if the process results can be replicated in large-scale controlled bioreactor counterparts through linear scale-up. It is evident that under these operating conditions, both cell lines demonstrate predictable growth and linear scale-up, maintaining cells/mL concentrations and nearly identical overall specific growth rates between the 0.1L and 3L bioreactor systems.

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**.

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