

Scalable Expansion of Human Pluripotent Stem Cells in Eppendorf BioBLU® 0.3 Single-Use Bioreactors

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Abstract

The routine application of human pluripotent stem cells and their derivatives in regenerative medicine and innovative drug discovery will require the constant supply of high cell numbers in consistent, high quality. Well monitored and controlled stirred-tank bioreactors represent suitable systems to establish up-scalable bioprocesses

enabling the required cell production. The following application note describes the successful cultivation of human pluripotent stem cells in suspension culture using Eppendorf BioBLU 0.3 Single-use Vessels in a DASbox® Parallel Mini Bioreactor System.

Introduction

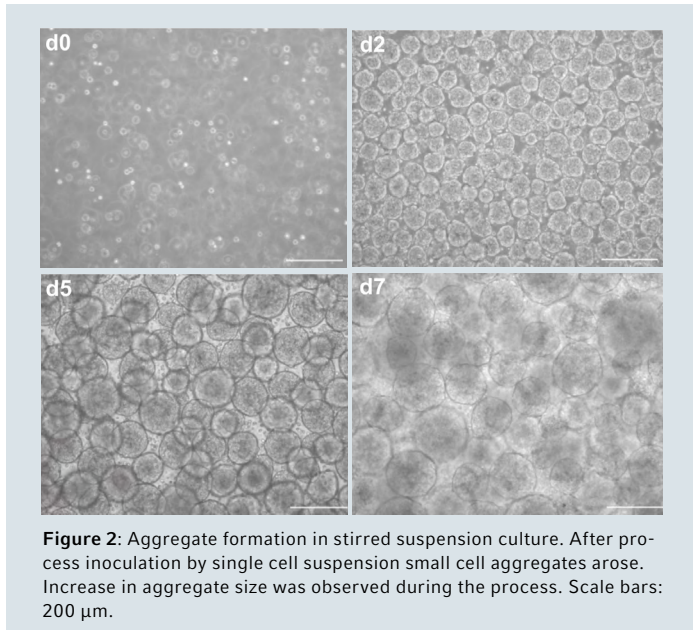
Human pluripotent stem cells (hPSCs), comprising human embryonic stem cells (hESC) and induced pluripotent stem cells (hiPSC), and their derivatives are considered promising cell sources for novel regenerative therapies [1]. Cell therapies aim at the replacement of cell or tissue loss induced by degenerative disorders such as cardiovascular and neurodegenerative diseases, diabetes and many others, which cannot be healed by currently established, conventional treatments. Moreover, specific human cell types derived from hPSCs by differentiation can be utilized for the development of yet unavailable *in vitro* disease models, novel drug discovery strategies and more predictive drug safety assays.

Most of the envisioned clinical and industrial applications will require billions of lineage-specific cells which cannot be produced by conventional surface-adherent 2-dimensional (2D) cultures. Stirred-tank bioreactors, which are widely used in the biopharmaceutical industry for the generation of recombinant proteins expressed in mammalian (tumor) cell lines, provide numerous advantages for process development, as they allow for online monitoring and

control of key process parameters such as pH, oxygen tension and biomass formation. Advanced bioreactor systems which have been developed in a wide range of culture vessels also facilitate the straightforward scale-up



Figure 1: DASbox Mini Bioreactor System with BioBLU 0.3 Single-use Vessels



to larger process dimensions. However, cultivation and differentiation of hPSCs in stirred bioreactors apparently require the adaptation of cell cultivation from the established 2D surface-adherent culture to 3-dimensional (3D) suspension culture. It was recently demonstrated that hPSCs can be successfully grown as free floating, "cell only aggregates" in small-scale suspension [2-4]. Based on this knowhow the transfer to a DASGIP® Parallel Bioreactor System with four individually controlled glass vessels having a working volume of 100 - 250 mL each, was established [5]. Optimization of stirring-controlled aggregate formation from single cell inoculated hiPSCs led to an approximately four-fold cell expansion resulting in 2×10^8 cells per vessel (100 mL) using a fed-batch process. However, with regard to the envisioned clinical application of hPSCs, the possibility to utilize single-use culture vessels, which will support the development of GMP-conform processes, is of great interest. Subsequently, aim of this work was to establish a suspension culture of hiPSCs in a parallel DASbox Mini Bioreactor System equipped with fully instrumented BioBLU 0.3 Single-Use Vessels.

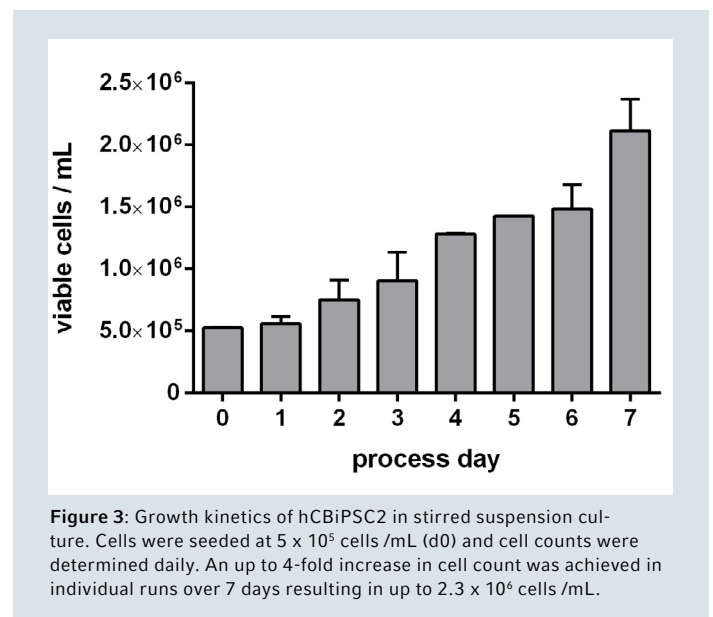
Materials and Methods

Experiments were performed utilizing the cord blood derived hiPSC line hCBiPSC2 [6]. Suspension cultures were initiated by detachment and dissociation of hiPSC monolayer cultures with accutase (Life Technologies). Single cells were suspended in mTeSR™1 (STEMCELL Technologies, Vancouver, Canada) supplemented with the ROCK inhibitor Y-27623 (10 μM). Each BioBLU 0.3 single-

use vessel was equipped with probes for pO_2 and pH. The pH probes were calibrated by two-point calibration. pO_2 probe calibration was conducted under process conditions: headspace gassing with 3 sL/h air plus 5% CO_2 , stirring at 70 rpm utilizing an pitched-blade impeller [5, 7], 37°C in 100 mL mTeSR™1; after stable pO_2 values were reached a slope calibration was performed. For culture inoculation 25 mL of a single-cell suspension were added to achieve a density of 5×10^5 cells /mL in the final 125 mL culture volume. After 48 h the entire medium was replaced daily (batch feeding) excluding cell loss. For cell counting and other analysis a sampling volume of 3.5 mL was harvested daily without medium replacement to prevent culture dilution. This strategy resulted in subsequent culture volume reduction from 125 to approximately 100 mL during the 7 day process duration. Beside pO_2 and pH, glucose and lactate concentrations, viable cell counts and the expression of pluripotency markers were monitored. Daily viable cell counts were performed via a trypan blue exclusion assay after cell-aggregate dissociation by collagenase B (Roche) treatment. Pluripotency assessment was performed by flow cytometry analysis specific to SSEA4 and TRA1-60.

Results and Discussion

24 h after inoculation of respective single cell suspensions to BioBLU 0.3 Single-Use Vessels small cell aggregates with an average diameter of $58.1 \pm 23.1 \mu\text{m}$ emerged in the stirred cultures. These aggregates, which showed a highly homogeneous size distribution throughout the process, increased in size over the cultivation period resulting in an average diameter of $139.25 \pm 25.37 \mu\text{m}$ (figure 2) on day 7.



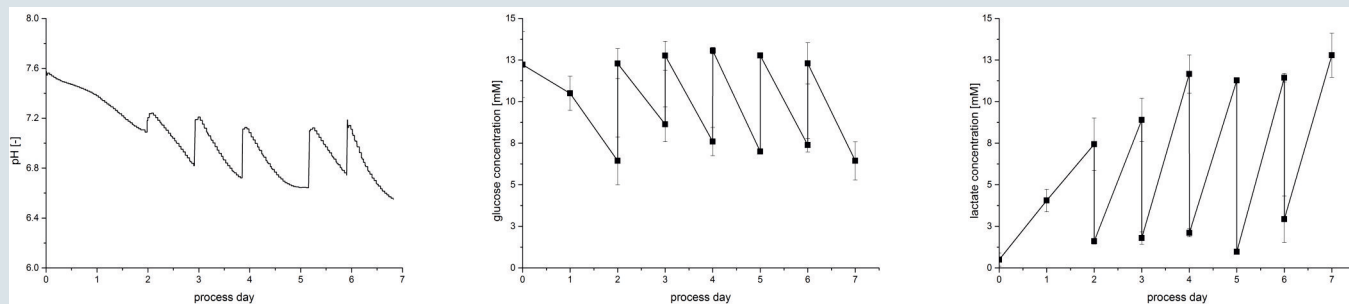


Figure 4: Metabolic activity of hCBiPSC2 in stirred suspension culture. The culture medium was replaced daily starting at 48 h thereby resulting respective glucose and lactate concentration patterns. The pH dropped from 7.4 in fresh medium to lowest values of ~6.8 at the end of a 24 h feeding interval on process days 5-7.

A robust ~4-fold increase in viable cell count was achieved in this fed-batch process resulting in an average cell concentration of 2.1×10^6 cells/mL on day 7 and thus a total cell yield of $\sim 2.1 \times 10^8$ cells per vessel (figure 3).

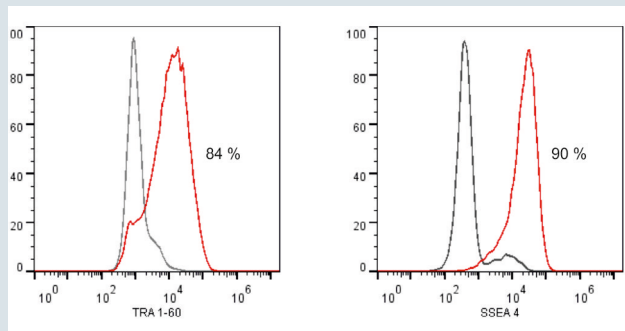


Figure 5: Expression of pluripotency-associated surface markers is retained after expansion in stirred suspension cultures. Flow cytometry revealed that the majority of cells that were harvested at the process endpoint (after day 7) expressed pluripotency-associated surface markers TRA 1-60 and SSEA 4 (gray line represents isotype controls).

Monitoring the metabolic activity revealed ~47% of glucose consumption and accumulation of 7.4 mM lactate at 48 hours. The metabolic activity was also followed by online measurements of pH and pO_2 . Increasing cell numbers over time resulted in a maximum pH drop to 6.8 (figure 4; as compared to pH 7.4 in fresh medium) and dissolved oxygen

levels decreased to 57% (data not shown). The expression of pluripotency-associated surface markers TRA 1-60 and SSEA4 were determined at the process endpoint to evaluate the quality of the expanded hPSCs. Flow cytometry revealed that the majority of the yielded cell population retained expression of these markers i.e. 84% positivity for TRA 1-60 and 90% for SSEA4 (figure 5) was observed suggesting maintenance of pluripotency in this cultivation process.

Conclusion

This set of experiments demonstrates the successful expansion of human pluripotent stem cells applying the DASbox system in combination with BioBLU 0.3 Single-Use Vessels. In a 7 day-lasting expansion process in stirred suspension culture cell yields of up to 2.3×10^8 cells /100 mL were obtained, which is in good agreement with our previous data in the DASGIP Parallel Bioreactor System, stirred glass vessel system (DS0200TPSS; 100-250 mL working volume) [5]. Notably cells generated by the described process retained expression of established, pluripotency associated cell surface markers. The work confirms the general applicability of the culture system for hPSC expansion in stirred suspension and reveals the DASbox system in combination with BioBLU 0.3 Single-Use Vessels to be an excellent platform for further process optimization and future adaptation to lineage-specific hPSC differentiation processes.

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