Pluripotent Stem Cell Expansion in a Stirred-Tank Bioreactor: Optimization and Automation of Feeding Strategies

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Introduction

Large-scale expansion of human pluripotent stem cells (hPSCs) is a rapidly evolving field with promising applications in cell therapy and regenerative medicine. Despite significant advances, economic viability remains challenging, hampering translation, largely driven by the high cost of cell culture media. Stirred-tank bioreactor technology represents an ideal platform for large-scale expansion, as it enables scalable operation with integrated monitoring and automation, supports high cell densities, and better recapitulates a 3D culture environment.

In this work, we describe two scalable suspension workflow strategies that support the expansion of hPSCs as free-floating, scaffold-free aggregates: (1) a high-density expansion scheme in a perfusion-fed BIOne single-use bioreactor (SUB, Distek), and (2) a parallel expansion process using DASbox Mini bioreactor (Eppendorf). Central to this work is HiDef® S8®, a new chemically defined, animal-free suspension medium developed specifically for the efficient expansion and maintenance of human pluripotent stem cells.

Materials and Methods

BIOne SUB (Fig. 1A) experiments were conducted in collaboration with Distek to evaluate HiDef-S8. DASBox Mini (Fig. 1B) studies compared four culture media for hPSC expansion: mTeSR1, E8, HiDef-B8 and HiDef-S8, performed by scientists in collaboration with IST. Daily samples were drawn from the bioreactors for cell counting, viability measurements, aggregate size and pluripotency assessment.

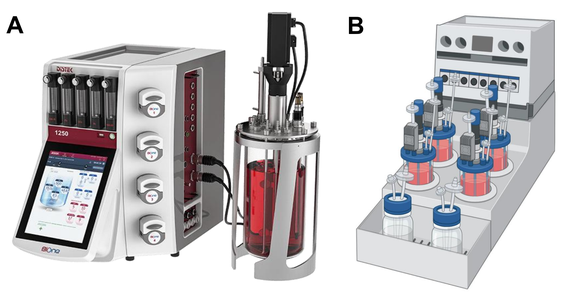

Figure 1. Suspension culture systems. BIOne single-use bioreactor (A) and DASbox Mini bioreactor (B).

Parameter	BIOne SUB	DASBox Mini
Seeding density	100,000 hPSCs / mL	
Working volume	80 mL	1,200 mL
Agitation	250 rpm	80 rpm
Media exchange	Continuous perfusion	

Table 1. Operational parameters for suspension culture

Results

HiDef-S8's growth rate was significantly faster than when using other culture media – 39x in 6 days (Fig. 2A). Only mTeSR1 and HiDef-S8 preserved high expression of key pluripotency markers (>95%) while maintaining viability (Fig. 2B). hPSCs expanded in HiDef-S8 produced aggregate diameters below 400 µm and readily differentiated into all three germ layers in adherent culture post-3D expansion (Fig. 2C).

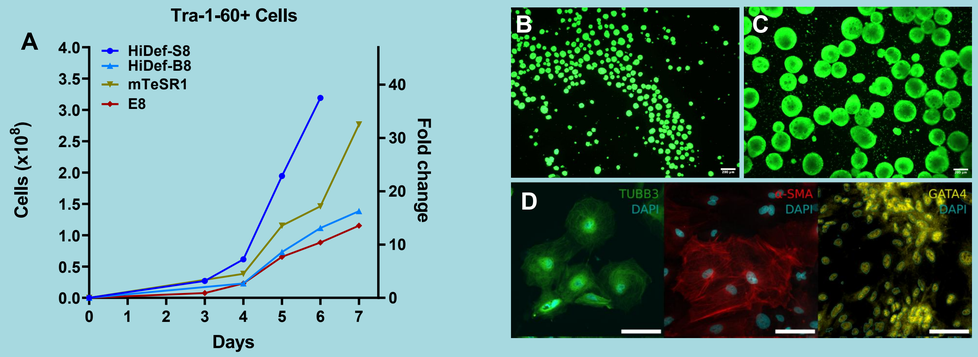

Figure 2. DASBox Mini suspension culture. Fold change of hPSCs using different cell culture media (A). Calcein AM of HiDef-S8 aggregates on Day 1 and Day 7 (B-C). Differentiation of HiDef-S8 derived aggregates (C).

Over the five days of suspension culture, the number of cells increased exponentially, resulting in a 60-fold expansion - 7.23 x 10^9 cells (Fig. 3A) with viable aggregates with diameters under 200 µm. The efficiency of cells produced was also higher in 3D culture vs 2D and reached approximately 1.5 million cells generated per mL of medium consumed (Fig. 3B).

3D aggregates were able to be replated back to 2D using HiDef-B8 (Fig. 4A). Quantification of pluripotency markers using flow cytometry demonstrated that replated hPSCs co-expressed surface markers Tra-1-60 and SSEA-4 at unchanged levels (Fig. 4B). Additionally, hPSCs were successfully differentiated to cardiomyocytes in 2D using the CDM3 protocol (Fig. 4C).

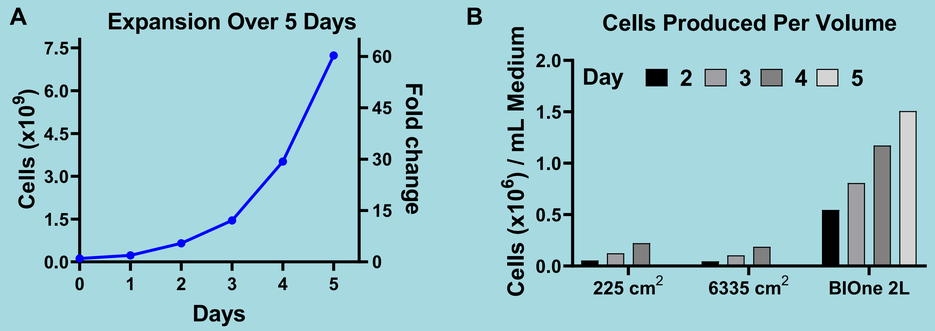

Figure 3. BIOne Mini suspension culture. Expansion of hPSCs (A) and efficiency of cells produced (B).

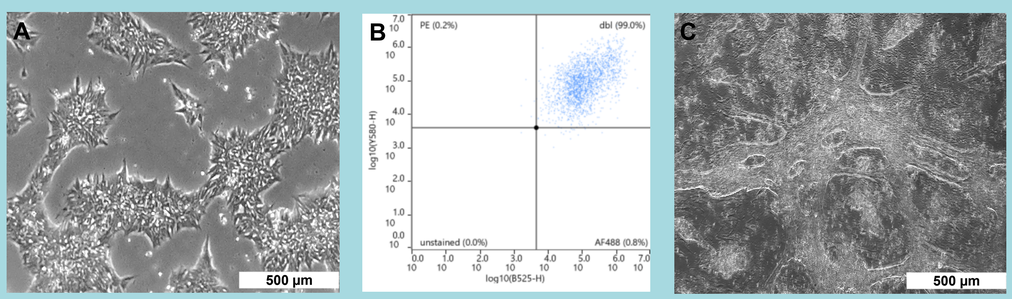

Figure 4. Pluripotency analysis. Aggregate morphology (A) and flow cytometry analysis (B) of suspension cultures in HiDef-S8 (Day 5). Cardiomyocyte differentiation on Day 18 (C).

Conclusions

HiDef-S8 achieved a 39-fold expansion of hPSCs within six days in the DASBox Mini bioreactor. While HiDef-S8 and mTeSR1 showed comparable performance in terms of TRA-1-60 cell yields, HiDef-S8 reduced culture media costs by 34% and supported a faster, stable growth rate, resulting in a significantly lower medium cost per cell produced.

In the BIOne SUB system, approximately 7 billion cells were generated within five days in a 2 L single-use bioreactor. The expanded hPSCs readily reattached in 2D adherent culture, exhibited normal morphology, retained pluripotency through multiple passages, and successfully differentiated into cardiomyocytes.

HiDef-S8 was specifically developed to address the demands of large-scale, high-density suspension culture. Its chemically defined, animal-origin-free formulation provides a robust and scalable platform for transitioning hPSC workflows from conventional adherent culture to high-efficiency bioreactor-based expansion. HiDef-S8 reduces cost per cell, improves medium utilization efficiency, and supports the quality requirements of both research and translational applications.

Future Work

Future work will focus on manufacturing cGMP-compliant HiDef-S8 to support clinical and commercial applications. Defined Bioscience aims to expand its 3D culture platform to enable the differentiation of hPSCs in suspension to further enhance scalability and efficiency for large-scale cell production.

References

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Acknowledgments

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