Stirred Suspension Culture of Human Pluripotent Stem Cells in mTeSR™1 Medium

Jennifer Antonchuk[1], Mark Fairey[1], Andrew Booth[1], Terry E. Thomas[1], Allen C. Eaves[1,2], and Sharon A. Louis[1]

[1] STEMCELL Technologies Inc, Vancouver, BC, Canada
[2] Terry Fox Laboratory, BC Cancer Agency, Vancouver, BC, Canada

Introduction

Human pluripotent stem cells (hPSCs), including embryonic and induced pluripotent stem cells (hESCs and hiPSCs) have traditionally been cultured in adherent 2-dimensional (2D) systems. As an example, hPSCs can be maintained long-term in mTeSR™1 defined, serum-free medium on plates pre-coated with Matrigel® or StemAdhere™ Defined Matrix for hPSC. Although feeder-free systems have alleviated much of the variability associated with hPSC culture, there remains an inherent limitation with 2D systems in the maximum cell yield being constrained by the available surface area of the cultureware used. In order to meet the demand for very high yields of cells required for applications such as drug screening using hPSC-derived cells and development of cellular therapies for regenerative medicine, high cell density 3D suspension culture systems would be required. Suspension culture systems have recently been described for the culture of hPSCs, including a method of seeding single hPSCs into mTeSR™1 medium where they will self-aggregate, and then culturing the aggregates with or without continuous stirring[1]. The goal of our study was to replicate the published system for suspension culture of hPSC aggregates using mTeSR™1 medium, to gain a better understanding of the system requirements, efficiencies, and ability to maintain pluripotency.

Methods

Static Suspension Culture

For static suspension culture, 10^5, $5x10^5$, or 10^6 H1 hESCs were seeded per well into uncoated 6-well plates in 3mL of mTeSR™1 medium supplemented with 10uM Y27632. Media was changed daily by harvesting the entire contents of the well, allowing cells and aggregates to settle to the bottom of a test tube, and replacing the media with fresh mTeSR™1 (without Y27632). After 4 days, cultures were passaged by harvesting the entire contents of the well, dissociating to single cells with Accutase® for 10 mins, performing cell counts, and reseeding with the original input cell number (10^5, $5x10^5$, or 10^6 cells/well).

Stirred Suspension Culture

For stirred suspension culture, H1 hESCs were seeded into 25mls of mTeSR™1 medium supplemented with 10uM Y27632 in Erlenmeyer flasks at densities of $3.3x10^4$ or $1.0x10^5$ cells/ml, and the flasks were placed onto a rotating shaker in the incubator. Cultures were incubated at 37°C, 5% CO_2, with 40rpm constant rotation, for 4 days with daily media changes of mTeSR™1. After 4 days, cultures were passaged as above, and reseeded at the original input cell density ($3.3x10^4$ or $1.0x10^5$ cells/ml).

Analyses

At the end of the culture period, cultures were assessed for maintenance of pluripotency by flow cytometry for expression of pluripotency markers SSEA3 and Oct4, as well as retention of undifferentiated morphology after replating onto Matrigel®-coated plates. For this, cells harvested from the suspension cultures were plated onto Matrigel®-coated plates, allowed to reach semi-confluency, and then passaged as clumps onto fresh Matrigel®-coated plates to microscopically assess both colony and cellular morphology.

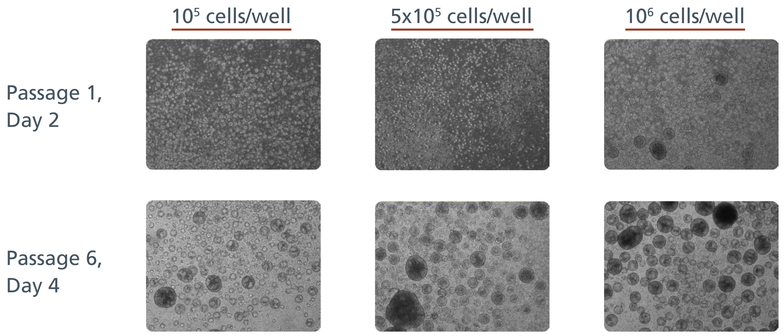
Conclusions

- H1 hESCs can be cultured for at least 12 passages in static suspension culture, or at least 8 passages in stirred suspension culture in mTeSR™1 medium
- Expansion rate increases as cells become adapted to the suspension culture system, and can reach 10-fold per 4-day passage
- hESCs maintain pluripotency, as assessed by expression of pluripotency markers SSEA3 and Oct4, or by cellular and colony morphology

[1] Zweigerdt, R., *et al.*, *Scalable expansion of human pluripotent stem cells in suspension culture.* Nat. Protocols, 2011. 6(5): p. 689-700.

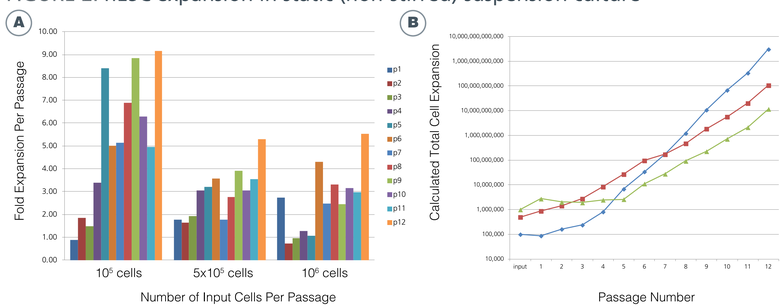
Static Suspension Culture

FIGURE 1: Static (non-stirred) suspension culture of hPSCs



Aggregates formed spontaneously within 24 hrs of seeding, and grew in size during the 4 day culture period. All images at 40x magnification.

FIGURE 2: hESC expansion in static (non-stirred) suspension culture

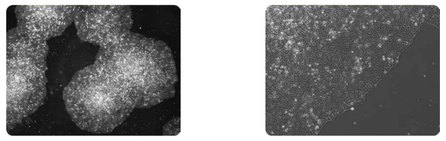


A) At the end of each 4-day culture period, cell were harvested and counted to determine fold expansion (n=1). Expansion rate increased with subsequent passages, especially when plated at lower densities. B) Total cumulative cell expansion was calculated from the fold expansion to show the rate at which high cell yields could theoritically be achieved if all cells were retained.

TABLE 1: Pluripotency marker expression after 12 passages in static suspension culture

Number of Cells Seeded / Passage	% SSEA3⁺	% Oct4⁺
10^5 cells	95.2	92.6
$5x10^5$ cells	90.1	95.7
10^5 cells	95.2	92.6

FIGURE 3: Maintenance of undifferentiated morphology in static suspension culture



hESCs grown in suspension for 12 passages retained the typical undifferentiated morphology when replated onto Matrigel®. Colonies were round, with phase-bright centres, and cells were tightly packed with prominent nucleoli and scant cytoplasm, as is typical of 2D mTeSR™1 cultures.
Left: 20x, Right: 100x

Stirred Suspension Culture

FIGURE 4: Stirred suspension culture of hPSCs

H1 hESCs were grown in mTeSR™1 medium in Erlenmeyer flasks, and the flasks were placed onto a rotating shaker at 37°C, 5% CO_2, with 40rpm constant rotation, for 4 days with daily media changes of mTeSR™1.

FIGURE 5: hESC expansion in stirred suspension culture

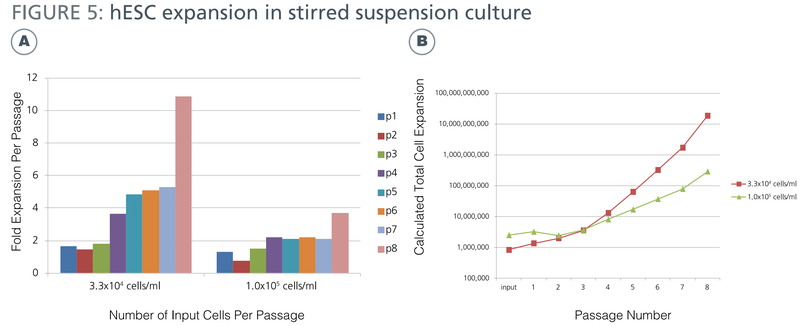


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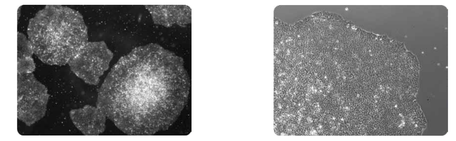
TABLE 2: Pluripotency marker expression after 8 passages in stirred suspension culture

Seeding Cell Density	% SSEA3⁺	% Oct4⁺
0.33 e5/mL	90.9	94.5
1.00 e5/mL	92.6	91.2

FIGURE 6: Maintenance of undifferentiated morphology in stirred suspension culture



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Left: 20x, Right: 100x