Robust Workflows for the Expansion and Differentiation of Human Pluripotent Stem Cells as Aggregates in Suspension Culture

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INTRODUCTION

STEMCELL’s portfolio of TeSR™ 3D-based media products have been developed for robust and scalable suspension culture of human pluripotent stem cells (hPSCs) as aggregates. However, the field has been challenged by the lack of methods that can reproducibly scale hPSCs cultures without prior adaptation of cells to higher biomass levels imposed in stirred suspension. A critical balancing act exists between the aggregation rate that maintains aggregates in suspension and the generation of shear. Agitation methods were tested for their ability to maintain aggregate suspensions and cell growth rates. Experiments were conducted with 6 different cell lines (3 PSC and 3 iPSC) in which cells were serially expanded in suspension culture up to 500 mL. Cells exhibit a sustained daily expansion of 7 cell lines in culture vessels tested from 2019 - present. Robust vessels outlined in orange having consistent daily fold expansion above 1.2. (B) Consistent expansion of hES and iPSC lines as aggregates in TeSR™-AOP 3D across multiple culture vessels. Greater than 10⁹ cells produced after 5 passages in suspension culture with no adaptation passage.

METHODS

(A) High-quality adherent hPSC cultures are dissociated non-enzymatically to clumps using Gentle Cell Dissociation Reagent (GCDR; Catalog #100-0074), with harvest on days 10, 17 and 24.

(B) After 3 or 4 days, aggregates are recovered using a 37 μm strainer (Catalog #27215/27250), then incubated in GCDR at 37°C for 6 minutes. GCDR is removed and aggregates are resuspended in seed medium + 10 μM Y-27632 (Catalog #72302). Fed-batch feed supplement is added on days 1 and 2, starting 24 hours after inoculating cell clumps.

(C) A half-medium change is performed on day 3 of a 4-day passage.

(D) Cells clumps are resuspended at 0.5 - 1 x 10⁵ viable cells/mL in seed medium + 10 μM Y-27632 (Catalog #72302). Fed-batch feed supplement is added on days 1 and 2, starting 24 hours after inoculating cell clumps. A half-medium change is performed on day 3 of a 4-day passage.

Aggregates were passaged non-enzymatically by dissociation using Gentle Cell Dissociation Reagent and filter-based trituration. The only system that gave reproducible growth across cell lines had a low-shear Vertical-Wheel® impeller design. With this workflow, hPSCs underwent a greater than 1.5- to 1.9-fold expansion per day (cell line dependent) with > 85% viability, > 90% expression of OCT4 and TRA-1-60, the capacity to differentiate to the three germ layers, and a normal karyotype. To verify the utility of this workflow, 3 hPSC lines were further differentiated into polyploid megakaryocytes (MKs) in 3D suspension cultures. Differentiation used established 2D protocols with a 12-day endothelial-to-hematopoietic transition phase, and a 5-day progenitor-to-mature MK stage. At the end of the protocol, 48% - 75% of cells expressed CD41a, 25% - 65% of the cells co-expressed CD41a and CD42b, and 10 - 60 CD41a+CD42b+ cells were generated per seeded hPSC (n = 9). The DNA ploidy profile of the CD41a+CD42b+ cells generated showed 26% and 9% of cells had 4N and 8N+ DNA ploidy, respectively. The combination of TeSR™ 3D workflows and low-shear bioreactors provides a robust system suitable for the expansion of a wide range of hPSC lines.

Flexible Family of Fed-Batch Optimized Media

3D Suspension Media Design Objectives

- No apoptosis step required from 3D to 3D suspension culture
- Cells exhibit a sustained daily expansion
- Fed-batch protocols to minimize culture handling and disruption
- Non-enzymatic filter-based passaging
- Culture viabilities at the end of each passage > 85%
- Protocols maintain high glucose and low lactate concentrations
- After 5 passages in suspension: equivalent expression of markers of undifferentiated hPSCs, functional pluripotency as measured by trimming differentiation, stable karyotype
- Lower cost per cell produced compared to traditional 2D culture media

RESULTS

FIGURE 1. Optimizing Suspension Culture Operation

(A) Daily fold expansion of 7 cell lines in culture vessels tested from 2019 - present. Robust vessels outlined in orange having consistent daily fold expansion above 1.2. (B) Good and ‘Bad’ aggregate morphology in different culture environments. Optimal aggregates are uniform in size and below 500 μm in diameter at day 4 of culture. They have a dished morphology in bright-field images resulting from multiple small voids distributed throughout the aggregate. (C) The immunofluorescent image shows an optically cleared corotational cross-section through a typical FSC aggregate showing these void spaces (blue:DAPI; green:OCT4). (D) Cells can be readily scaled up from 2D cultures to 2 mL suspension cultures in 6-well plates, from there up to 30 mL in an orbital shaker bottle, and then into 100 and 500 mL cultures in PBS-MINI Bioreactors (Catalog #100-1006 and #100-1007, respectively).

FIGURE 2. Reproducible growth in the PBS-MINI Bioreactors

(A) Daily fold expansion for hES and iPFS aggregates in mTeSR™3D and TeSR™-AOP 3D in the PBS-MINI alongside filter bottle control cultures. (B) Consistent expansion of hES and iPFS cell lines as aggregates in TeSR™-AOP 3D across multiple culture vessels. Greater than 10⁹ cells produced after 5 passages in suspension culture with no adaptation passage.

FIGURE 3. Hematopoietic Differentiation in Suspension Culture

(A) The number of CD41a+ cells generated per input hPSC and (B) the frequency of CD41a+CD42b+ MKs after culturing hPSCs for 12 and 17 days in suspension culture using the STEm3D Megakaryocyte Kit. (C) Morphology evolution of the MK aggregates over the first 12 days and of the reseeded cells. (D) Frequency of GlyA+CD42b+ erythroblasts and (E) the number of GlyA+ erythroblasts generated per input hPSC after culturing for 24 days in suspension culture using the STEm3D Erythroid Kit.

CONCLUSION

- Robust workflows for the expansion and differentiation of human pluripotent stem cells as aggregates in suspension culture
- High-quality adherent hPSC cultures are dissociated non-enzymatically to clumps using Gentle Cell Dissociation Reagent (GCDR; Catalog #100-0074), with harvest on days 10, 17 and 24.
- A half-medium change is performed on day 3 of a 4-day passage.
- Cells clumps are resuspended at 0.5 - 1 x 10⁵ viable cells/mL in seed medium + 10 μM Y-27632 (Catalog #72302). Fed-batch feed supplement is added on days 1 and 2, starting 24 hours after inoculating cell clumps. A half-medium change is performed on day 3 of a 4-day passage.
- Cells exhibit a sustained daily expansion
- Fed-batch protocols to minimize culture handling and disruption
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