Cells can be scaled up to ~ 1 x 10^9 cells in 4 - 5 passages. Cells typically expand at ~1.3 - 1.6-fold per day. With this passaging schedule, ~ 1 x 10^9 can be produced in a final volume of 2500 mL in shaker or spinner flasks. Expansion rates of hPSCs cultured as aggregates ranged from 1.3 to 1.6-fold per day (dependent on cell line) with consistent maintenance of pluripotent stem cell marker expression (OCT4) and trilineage differentiation potential at all scales.

Materials and Methods

Figure 1. Process Scale-up for Production of 1 x 10^9 hPSCs in Aggregate Suspension Culture using mTeSR™3D

(A) High quality 2D cultures are dissociated to clumps using non-enzymatic Gentle Cell Dissociation Reagent (GCDR, STEMCELL); (B) Cells are resuspended (1 - 2 x 10^6 cells/mL) in mTeSR™3D Seed Medium with 10 µM Y-27632 ROCK inhibitor; (C) Cells can then be cultured in low adherence plates or unbaffled cylindrical orbital shake flasks. Spinner flasks or bioreactors can also be used; mTeSR™3D Feed Medium is added daily starting 24 hours after inoculating cells; (D) After 3 or 4 days, hPSC aggregates are recovered using a 37 µm Reversible Strainer (STEMCELL), and dissociated to clumps using GCDR; (E) Cells are seeded into a clean vessel in mTeSR™3D 3D Seed Medium with 10 µM Y-27632 ROCK inhibitor; (F) mTeSR™3D employs a fed-batch feeding strategy; required nutrients are added daily without the need for a medium exchange.

Results

Figure 3. Identification of Mixing Conditions for hPSC Growth in Orbital Shake Flasks

(A) Initial empirical studies sought to identify the mixing conditions that provide optimal growth of hPSCs cultured as aggregates. A range of culture volumes, mixing speeds (rpm) and orbital diameters were tested. Typical results for a 300 mL cylindrical flask are shown. The optimal mixing rpm occurred over a relatively narrow range; if mixing is insufficient, cell aggregates will merge into a large flattened cluster of cell aggregates; if mixing is sufficient to fully suspend cell aggregates in the culture volume, shear effects inhibit growth and lead to poor expansion or cell death. It was determined that the appropriate mixing speed for any given culture volume, vessel diameter and orbital throw diameter, would just lift the cell aggregates from the bottom of the vessel without fully mixing the aggregates into suspension. (B) The degree of mixing can be visualized using appropriate tracer particles for any given bottle diameter, culture volume and orbital diameter. Here CytoPlex 3 microcarrier particles (Sigma) are used to visualize the degree of mixing that supports hPSC aggregate cultures in orbital shake flasks.

Conclusions

- mTeSR™3D provides a convenient method using fed-batch feeding protocols to scale up cultures of undifferentiated hPSCs as aggregates in suspension.
- Orbital shaker cultures can be used to economically scale up to 1 x 10^9 hPSCs in as little as 5 passages in aggregate suspension culture using mTeSR™3D.
- hPSC aggregates expanded in mTeSR™3D and passaged with GCDR maintained expression of OCT4 and efficiently differentiated into the 3 germ layers using the STEMdiff™ TriLineage Differentiation Kit. Cells cultured as aggregates readily differentiate into the three germ layers with high efficiency.