PANCREATIC CELLS DERIVED FROM HUMAN PLURIPOTENT STEM CELLS, which are herein incorporated by reference in their entireties. Critical for the progression of hESC-based technology to clinical trials is a demonstration of comparable scalability. Improvements that enhance expansion efficiencies will also save time and produce cost savings, as well as minimize the potential for population drift over time spent in culture. *See* Maitra *et al.* 2005 *Nat Genet* 37:1099-1103, which is incorporated herein by reference in its entirety. Importantly, scaling using roller bottles as described herein, for example, along with cryopreservation of hESC, provides a defined and consistent material for product manufacture for near and long term research and development strategies.

SUMMARY

In one aspect, there is provided a roller bottle comprising primate pluripotent stem cell (pPSC) aggregates in suspension in a physiologically acceptable medium, wherein the pPSC aggregates are formed in the roller bottle.

In another aspect, there is provided a method for preparing the roller bottle of the present, comprising:

a. seeding a roller bottle with a single cell suspension of undifferentiated pPSCs in a culture medium that supports undifferentiated growth of the pPSCs; and

b. agitating the single cell suspension at low rotation speed in a roller bottle to form pPSC cell aggregates, thereby preparing a roller bottle comprising pPSC aggregates in suspension in a physiologically acceptable medium.

[0023] The disclosure relates to compositions comprising a basal salt nutrient solution and an ErbB3 ligand, with the compositions being essentially free of serum.

[0024] The disclosure also relates to compositions comprising a basal salt nutrient solution and a means for stimulating ErbB2-directed tyrosine kinase activity in differentiable cells.

[0025] The disclosure relates to methods of culturing differentiable cells, with the methods comprising plating the differentiable cells on a cell culture surface, providing a basal salt nutrient solution to the differentiable cells and providing a ligand that specifically binds ErbB3.

[0026] The disclosure relates to methods of culturing differentiable cells, with the methods comprising plating the differentiable cells on a cell culture surface and providing a basal salt nutrient solution to the differentiable cells and a means for stimulating ErbB2-directed tyrosine kinase activity in the differentiable cells.

[0027] The disclosure also relates to methods of culturing differentiable cells, with the methods comprising providing a digest solution to a layer of differentiable cells that are contained in a culture chamber prior to digestion, where the digestion breaks apart the layer of cells into single cells. After digestion, the single cells are placed into a new tissue culture chamber with a differentiable cell culture solution, wherein the differentiable cell culture solution comprises a basal salt nutrient solution and an ErbB3 ligand. Once cultured, the single differentiable cells are placed in conditions that permit growth and division of the single cells.

[0028] The disclosure relates to methods for generating a hES cell aggregate in suspension from a pluripotent hES adherent culture, by culturing a hES cell in an adherent growth culture condition which allows for expansion in an undifferentiated state; disassociating the adherent hES cell culture into a single cell suspension culture; contacting the single cell suspension culture with a first differentiating culture condition which allows for formation of hES-derived cell aggregates in suspension by agitating the single cell suspension culture until such a period of time when the single cell suspension culture forms a hES-derived cell aggregate in suspension, and thereby generating a hES-derived cell aggregate in suspension. In some examples, agitation of the single cell suspension culture is performed by rotation at about 80 rpm to 160 rpm

[0029] The disclosure also relates to methods for generating a hES-derived cell aggregate in suspension from a hES-derived single cell suspension, by culturing a hES cell in an adherent growth culture condition which allows for expansion in an undifferentiated state; contacting the undifferentiated hES cell with a first differentiating culturing condition suitable for differentiating the hES cell and resulting in an adherent hES-derived cell; disassociating the adherent hES-derived cell into a single cell suspension culture; contacting the single cell suspension culture with a second differentiating culture condition which allows for formation of hES-derived cell aggregates in suspension by agitating the single cell suspension culture until such a period of time when the single cell suspension culture forms a hES-derived cell aggregate in suspension, and thereby generating a hES-derived cell aggregate in suspension. In some examples, agitation of the single cell suspension culture is performed by rotation at about 80 rpm to 160 rpm.

[0030] The disclosure relates to a roller bottle containing primate pluripotent stem cell (pPSC) aggregates in suspension. In certain example, the pPSC aggregates are cells selected from the group consisting of human embryonic stem cells (hESC), induced pluripotent stem cells (iPSC) and/or other human pluripotent stem cells. In one example, the roller bottle is not vented, but can be vented depending on the incubator or oven capabilities.

[0031] The disclosure also relates to methods for generating a roller bottle containing pPSC aggregates by contacting pPSCs with a pluripotent stem cell culture condition, and agitating the culture until pPSC aggregates form, thereby generating pPSC aggregates in the roller bottle. In certain examples, agitation of the pPSC culture is performed by rotation at about 3 rpm, about 4 rpm, about 5 rpm, about 6 rpm, about 7 rpm, about 8 rpm, about 9 rpm, about 10 rpm, about 11 rpm, about 12 rpm, about 13 rpm, about 14 rpm, about 15 rpm, about 16 rpm, about 17 rpm, about

18 rpm, about 19 rpm, about 20 rpm, about 21 rpm, about 22 rpm, about 23 rpm, about 24 rpm, about 25 rpm, about 26 rpm, about 27 rpm, about 28 rpm, about 29 rpm and about 30 rpm. Typically, agitation of the pPSC culture is performed by rotation at about 5 rpm, about 6 rpm, about 7 rpm, about 8 rpm, about 9 rpm, about 10 rpm, about 11 rpm, and about 12 rpm.

[0032] Another example of the disclosure relates to methods for differentiating pPSC aggregates in a roller bottle by contacting differentiable or undifferentiated pPSC aggregates with a culturing condition that differentiates the pPSCs, and agitating the pPSC aggregate culture until formation of pPSC-derived aggregates, thereby generating pPSC-derived aggregates in suspension in a roller bottle. In certain examples, agitation of the pPSC-derived aggregates suspension culture is performed by rotation at about 3 rpm, about 4 rpm, about 5 rpm, about 6 rpm, about 7 rpm, about 8 rpm, about 9 rpm, about 10 rpm, about 11 rpm, about 12 rpm, about 13 rpm, about 14 rpm, about 15 rpm, about 16 rpm, about 17 rpm, about 18 rpm, about 19 rpm, about 20 rpm, about 21 rpm, about 22 rpm, about 23 rpm, about 24 rpm, about 25 rpm, about 26 rpm, about 27 rpm, about 28 rpm, about 29 rpm and about 30 rpm. Typically, agitation of the pPSC culture is performed by rotation at about 5 rpm, about 6 rpm, about 7 rpm, about 8 rpm, about 9 rpm, about 10 rpm, about 11 rpm, and about 12 rpm.

[0033] Still another example of the disclosure relates to methods where fluid flow within a rolling bottle type of vessel involves rolling movement that does not require rotation or rolling the bottle. In one example, the rolling type movement is substantially re-created but without the use of a rolling vessel. In another example, a primate pluripotent stem cell culture has imparted fluid movement, for example, by pumping or flowing a fluid in a smooth, orderly manner with little or no turbulence. In such examples, any sub-current generally moves in parallel with any other nearby sub-current(s). This type of movement is also characterized as laminar flow (commonly used to move viscous fluids, especially those moving at low velocities) or streamline flow (a steady movement of fluid movement). In a yet another example, the fluid movement involves one or more baffles, which distribute the fluid flow within a chamber to create a continuous, uniform suspension of cells. In a still further example, the fluid movement involves one or a combination of deflector plates, distribution channels, and/or flow channels. In each example, there is included at least one or more seals on the culture vessel to ensure an aseptic environment inside the vessel during cell aggregation, growth and differentiation.

[0034] The disclosure also relates to methods for enriching or varying the composition of the resulting cell culture and/or population of an hES-derived cell aggregate suspension by optimizing the cell density of the pluripotent cell cultures or varying the concentration of various growth factors, for example, FGF10, EGF, KGF, noggin and retinoic acid, apoptotic inhibitors, Rho-kinase inhibitors and the like.

BRIEF DESCRIPTION OF THE DRAWINGS

[0035] FIGURE 1 depicts real time RT-PCR expression analysis of ADAM19, Neuregulin1, and ErbB1-3 in BG01v grown in defined conditions (8 ng/mL FGF2, 100 ng/mL LR-IGF1, 1 ng/mL Activin A). GAPDH and OCT4 control reactions are indicated.

[0036] FIGURE 2 depicts the inhibition of proliferation of BG01v cells using AG879. BG01v cells were plated in 6-well trays and exposed to DMSO (A), 50 nM-20 μM AG1478 (B), or 100 mM-20 μM AG879 (C) 24 hours after plating. After 5 days in culture, the cultures were fixed and stained for alkaline phosphatase activity. AG1478 did not appear to affect proliferation at these concentrations (20 μM shown in B), but AG879 substantially slowed cell growth at 5 μM (C).

[0037] FIGURE 3 depicts the morphology of BG01v cells cultured in DC-HAIF, which is defined culture media containing 10 ng/mL HRG-β, 10 ng/mL Activin A, 200 ng/mL LR-IGF1 and 8 ng/mL FGF2 (A and B), and in defined culture media (DC) containing 10 ng/mL HRG-β, 10 ng/mL Activin A, and 200 ng/mL LR-IGF1 (C and D).

[0038] FIGURE 4 depicts the expression of ADAM19, Neuregulin1, and ErbB1-4 by RT-PCR in mouse ES cells (A) and MEFs (B).

[0039] FIGURE 5 depicts the inhibition of ErbB1 and ErbB2 signaling in mouse ES cells. $2x10^5$ Mouse R1 ES cells were plated on 1:1000 MATRIGEL™ in 10% FBS, 10% KSR with 1000 U/mL mouse LIF (ESGRO). The following day, DMSO (carrier control), 1-50 μM AG1478, or 1-50 μM AG879 was added with fresh medium. The cultures were fixed on day 8, and stained for alkaline phosphatase activity. DMSO (A) and 1-50 μM AG1478 (B and C) did not overtly inhibit proliferation. AG879 substantially inhibited cell growth at 50 μM (compare D and F) and may have slowed proliferation at 20 μM (E).

[0040] FIGURE 6 depicts the inhibition of proliferation of BG02 cells grown in conditioned media (CM). (A) 50 μM AG825 inhibited proliferation of BG02 hESCs growing in CM. (B) AG825