clinical safety and efficacy. While the best outcome would be the availability of chemically defined media for hESCs, components that are not chemically defined would be acceptable if they were produced to GMP standard. There is a need, therefore, to identify methods and compositions for the culture and stabilization of a population of pluripotent stem cells that are able to be used for therapeutic purposes, wherein the culture compositions are defined and/or produced to GMP standard.

SUMMARY ~~OF THE INVENTION~~

[0016] The present disclosure~~invention~~ relates to compositions comprising a basal salt nutrient solution and an ErbB3 ligand, with the compositions being essentially free of serum.

[0017] The present disclosure~~invention~~ also relates to compositions comprising a basal salt nutrient solution and a means for stimulating ErbB2-directed tyrosine kinase activity in differentiable cells.

[0018] The present disclosure~~invention~~ 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.

[0019] The present disclosure~~invention~~ 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.

[0020] The present disclosure~~invention~~ 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.

[0021] The present disclosure~~invention~~ 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 preferred embodiments, agitation of the single cell suspension culture is performed by rotation at about 80 rpm to 160 rpm

[0022] The present disclosure~~invention~~ 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 preferred embodiments, agitation of the single cell suspension culture is performed by rotation at about 80 rpm to 160 rpm.

[0023] The present disclosure~~invention~~ 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 and Rho-kinase inhibitors.

[0023a] In one aspect, there is provided a method of generating hES cell-derived cell aggregates in suspension from a hES cell single cell suspension culture, comprising: (a) culturing hES cells in an adherent growth culture condition which allows for expansion in an undifferentiated state; (b) disassociating the adherent hES cell culture of step (a) into a hES cell single cell suspension culture; (c) agitating the hES cell single cell suspension culture to form hES cell aggregates in suspension; and (d) contacting the hES cell aggregates in suspension with a differentiating culture condition, thereby generating hES cell-derived cell aggregates in suspension.

[0023b] In another aspect, there is provided a method of generating hES cell-derived cell aggregates in suspension from a hES cell-derived single cell suspension culture, comprising: (a) culturing hES cells in an adherent growth culture condition which allows for expansion in an undifferentiated state; (b) contacting the undifferentiated hES cells with a first differentiating culturing condition suitable for differentiating the hES cells and resulting in an adherent hES cell-derived cell; (c) disassociating the adherent hES cell-derived cell into a hES-derived single cell suspension culture; and (d) agitating the hES-derived single cell suspension culture to form hES-derived cell aggregates in suspension; thereby generating hES cell-derived cell aggregates in suspension.