CLAIMS

What is claimed is:

1. A roller bottle comprising a culture of primate pluripotent stem cell (pPSC) aggregates suspended in a physiologically acceptable medium.

2. The roller bottle of claim 1, wherein the pPSC aggregates have a diameter of about 100 to 300 microns

3. The roller bottle of claim 1, wherein the culture is substantially free of pPSC agglomerations having a diameter of more than 300 microns.

4. The roller bottle of claim 1, wherein the pPSC aggregates express at least one marker selected from the group consisting of OCT4, NANOG, SSEA-3, SSEA-4, Tra-1-80 and Tra-1-60.

5. The roller bottle of claim 1, wherein the pPSC aggregates are human cells.

6. The roller bottle of claim 5, wherein the pPSC aggregates are selected from the group consisting of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC).

7. The roller bottle of claim 1, wherein the primate pluripotent stem cell-derived cell aggregates in suspension are generated by a method comprising:

a. providing a culture of undifferentiated pPSCs;

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

c. agitating the pPSCs at low rotation speed to form pPSC aggregates,

thereby generating a roller bottle comprising a culture of pPSC aggregates.

8. The roller bottle of claim 7, wherein the pPSC aggregates have a diameter of about 100 to 300 microns.

9. The roller bottle of claim 7, wherein the culture is substantially free of pPSC agglomerations having a diameter of more than 300 microns.

10. The roller bottle of claim 7, wherein the pPSC aggregates express at least one marker selected from the group consisting of OCT4, NANOG, SSEA-3, SSEA-4, Tra-1-80 and Tra-1-60.

11. The roller bottle of claim 7, wherein the pPSC aggregates are human cells.

12. The roller bottle of claim 11, wherein the pPSC aggregates are cells selected from the group consisting of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC).

13. The roller bottle of claim 7, wherein the rotation speed ranges from 3 to 20 rpm.

14. The roller bottle of claim 7, wherein the rotation speed ranges from 5 to 12 rpm.

15. The roller bottle of claim 7, wherein the culture of undifferentiated pPSCs in step a is a suspension of single cell pPSCs.

16. A method for preparing the roller bottle of claim 1, comprising:

a. providing a culture of undifferentiated pPSCs;

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

c. agitating the single cell suspension at low rotation speed in a roller bottle to form pPSC cell aggregates,

thereby preparing a roller bottle comprising a culture of pPSC aggregates suspended in a physiologically acceptable medium.

17. The method of claim 16, wherein the culture of undifferentiated pPSCs is a single cell suspension of pPSCs.

18. The method of claim 16, wherein the culture of undifferentiated pPSCs is a suspension of thawed pPSCs suspended in a culture medium.

19. The method of claim 16, wherein the rotation speed is about 3 rpm to about 20 rpm.

20. The method of claim 16, wherein the rotation is about 5 rpm to about 20 rpm.

21. The method of claim 16, wherein the rotation speed is about 5 rpm to about 12 rpm.

22. The method of claim 16, wherein the pPSC is a human cell.

23. The method of claim 22, wherein the pPSC is selected from the group consisting of hESC and iPSC.

24. A method of differentiating primate pluripotent stem cells (pPSCs) in a roller bottle comprising:

a. providing a culture of undifferentiated pPSCs;

b. agitating the pPSCs in a physiologically acceptable medium in a roller bottle at low rotation speed to form pPSC aggregates; and

c. contacting the pPSC aggregates in the roller bottle with a culture medium that differentiates the pPSCs to form differentiated cell aggregates,

thereby differentiating pPSCs in a roller bottle.

25. The method of claim 24, wherein the pPSCs are differentiated in a feeder-, matrix- and adherent growth culture-free environment.

26. The method of claim 24, wherein the rotation speed is about 3 rpm to about 20 rpm.

27. The method of claim 24, wherein the rotation speed is about 5 rpm to about 20 rpm.

28. The method of claim 24 wherein the rotation speed is about 5 rpm to about 12 rpm.

29. The method of claim 24, wherein the pPSCs are human cells.

30. The method of claim 29, wherein the pPSCs are selected from the group consisting of hESCs and iPSCs.

CLAIMS

What is claimed is:

1. 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.

2. The roller bottle of claim 1, wherein the pPSC aggregates have a diameter of about 100 to 300 microns

3. The roller bottle of claim 1, substantially free of pPSC agglomerations having a diameter of more than 300 microns.

4. The roller bottle of claim 1, wherein the pPSC aggregates express at least one marker selected from the group consisting of OCT4, NANOG, SSEA-3, SSEA-4, Tra-1-80 and Tra-1-60.

5. The roller bottle of claim 1, wherein the pPSC aggregates are human cells.

6. The roller bottle of claim 5, wherein the pPSC aggregates are selected from the group consisting of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC).

7. The roller bottle of claim 1, wherein the pPSC aggregates in suspension are generated by a method comprising:

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

b. agitating the pPSCs at low rotation speed to form pPSC aggregates, thereby generating a roller bottle comprising pPSC aggregates.

8. The roller bottle of claim 7, wherein the pPSC aggregates have a diameter of about 100 to 300 microns.

9. The roller bottle of claim 7, substantially free of pPSC agglomerations having a diameter of more than 300 microns.

10. The roller bottle of claim 7, wherein the pPSC aggregates express at least one marker selected from the group consisting of OCT4, NANOG, SSEA-3, SSEA-4, Tra-1-80 and Tra-1-60.

11. The roller bottle of claim 7, wherein the pPSC aggregates are human cells.

12. The roller bottle of claim 11, wherein the pPSC aggregates are cells selected from the group consisting of human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC).

13. The roller bottle of claim 7, wherein the rotation speed ranges from 3 to 20 rpm.

14. The roller bottle of claim 7, wherein the rotation speed ranges from 5 to 12 rpm.

15. The roller bottle of claim 7, wherein the undifferentiated pPSCs in step a is a suspension of single cell pPSCs.

16. A method for preparing the roller bottle of claim 1, 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.

17. The method of claim 16, wherein the undifferentiated pPSCs is a suspension of thawed pPSCs.

18. The method of claim 16, wherein the rotation speed is about 3 rpm to about 20 rpm.

19. The method of claim 16, wherein the rotation is about 5 rpm to about 20 rpm.

20. The method of claim 16, wherein the rotation speed is about 5 rpm to about 12 rpm.

21. The method of claim 16, wherein the pPSC is a human cell.

22. The method of claim 21, wherein the pPSC is selected from the group consisting of hESC and iPSC.