[0019] For the purposes of regenerative medicine, there is a desire to develop methods that allow derivation and long-term cultures of pluripotent stem cells under chemically defined, xeno-free, pathogen-free, and stable batch-to-batch conditions. Moreover, such methods should allow fast and economically efficient scale-up to acquire large quantities of pluripotent hES/hiPS cells in a short period of time. Preferably, the methods should also allow clonal survival of human ES cells in media containing no synthetic inhibitor of apoptosis, that could facilitate scientific and clinical applications involving cell sorting or gene knock-out in the cells.

SUMMARY

[0020] The present disclosure provides isolated laminin-521 (also known as LN-521 or laminin-11) and methods for producing isolated laminin-521. In further examples, the present disclosure provides recombinant host cells that express laminin-521 chains and secrete recombinant laminin-521.

[0021] In other examples, the present disclosure provides GMP quality laminin-521 for culturing cells for differentiation and maintenance for the purpose of developing cells for human cell therapy. The present disclosure also provides pharmaceutical compositions, comprising isolated laminin-521 together with a pharmaceutically acceptable carrier. Such pharmaceutical compositions can optionally be provided with other extracellular matrix components.

[0022] The present disclosure also provides methods to effectively generate amounts of isolated laminin-521 for various uses. In preferred examples of those uses, recombinant laminin-521 is used. Kits comprising an amount of isolated laminin-521, or pharmaceutical compositions thereof, effective for the desired effect, and instructions for the use thereof, are also disclosed.

[0023] The present disclosure also provides methods for culturing stem cells in monolayer cultures which facilitates cellular homogeneity, removal of the stem cells from a cell culture plate or other cellular support in single cell suspension, and replating stem cells in single cell suspension for passaging and expansion in significant dilutions that enable expansion of stem cell cultures and large scale production of such cells.

[0023a] In one aspect, the present disclosure refers to a cell culture substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; wherein the laminin is laminin-521; and wherein the cadherin is e-cadherin.

[0023b] In another aspect, the present disclosure refers to a cell culture system, comprising: a cell culture medium comprising at least 0.3 mM of albumin; and a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; wherein the laminin is laminin-521, and wherein the cadherin is e-cadherin.

[0023c] In another aspect, the present disclosure refers to a cell culture system, comprising: mTeSR1 cell culture medium; and a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; wherein the laminin is laminin-521, and wherein the cadherin is e-cadherin.

[0023d] In another aspect, the present disclosure refers to a method for maintaining stem cells, comprising: plating the stem cells on a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; and

exposing the stem cells to a cell culture medium comprising at least 0.3 mM of albumin; wherein the laminin is laminin-521, and wherein the cadherin is e-cadherin.

[0023e] In another aspect, the present disclosure refers to a method for maintaining stem cells, comprising: plating the stem cells on a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; and

exposing the stem cells to a mTeSR1 cell culture medium; wherein the laminin is laminin-521, and wherein the cadherin is e-cadherin.

[0024] In further examples, the present disclosure describes culturing pluripotent stem cells on laminin-521 in monolayer culture, removing stem cells from the cell culture plates along with other cell supporting materials in single cell suspension,

CLAIMS:

1. A cell culture substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor;
wherein the laminin is laminin-521; and
wherein the cadherin is e-cadherin.

2. The substrate of claim 1, wherein the laminin is an effective recombinant laminin.

3. The substrate of claim 1, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

4. The substrate of claim 1, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

5. A cell culture system, comprising:
a cell culture medium comprising at least 0.3 mM of albumin; and
a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor;
wherein the laminin is laminin-521, and
wherein the cadherin is e-cadherin.

6. The system of claim 5, wherein the cell culture medium is mTeSR1 medium with additional albumin added to arrive at an albumin concentration of at least 0.3 mM.

7. The system of claim 5, wherein the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

8. The system of claim 5, wherein the albumin is human serum albumin.

9. The system of claim 8, wherein the human serum albumin is recombinant human serum albumin.

10. The system of claim 5, wherein the laminin is an effective recombinant laminin.

11. The system of claim 5, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

12. The system of claim 5, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

13. The system of claim 5, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

14. A cell culture system, comprising:

mTeSR1 cell culture medium; and

a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor;

wherein the laminin is laminin-521, and

wherein the cadherin is e-cadherin.

15. The system of claim 14, wherein additional albumin is added to arrive at a final albumin concentration of at least 0.3 mM.

16. The system of claim 14, wherein additional albumin is added to arrive at a final albumin concentration of 0.39 mM.

17. The system of claim 14, wherein the laminin is an effective recombinant laminin.

18. The system of claim 14, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

19. The system of claim 14, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

20. The system of claim 14, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

21. A method for maintaining stem cells, comprising:

plating the stem cells on a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; and

exposing the stem cells to a cell culture medium comprising at least 0.3 mM of albumin;

wherein the laminin is laminin-521, and

wherein the cadherin is e-cadherin.

22. The method of claim 21, wherein the cell culture medium is mTeSR1 medium with additional albumin added to arrive at an albumin concentration of at least 0.3 mM.

23. The method of claim 21, wherein the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

24. The method of claim 21, wherein the albumin is human serum albumin.

25. The method of claim 21, wherein the human serum albumin is recombinant human serum albumin.

26. The method of claim 21, wherein the laminin is an effective recombinant laminin.

27. The method of claim 21, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

28. The method of claim 21, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

29. The method of claim 21, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

30. The method of claim 21, wherein the stem cells are plated at a density of 200 cells / mm^2 or less.

31. The method of claim 21, wherein the stem cells are plated at a density of 200 cells / mm^2 or more.

32. The method of claim 21, wherein the stem cells are plated such that no two stem cells contact each other.

33. A method for maintaining stem cells, comprising:

plating the stem cells on a substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment, wherein a protein fragment contains one, two or three functional domains that possesses binding activity to another molecule or receptor; and

exposing the stem cells to a mTeSR1 cell culture medium;

wherein the laminin is laminin-521, and

wherein the cadherin is e-cadherin.

34. The method of claim 33, wherein additional albumin is added to arrive at a final albumin concentration of at least 0.3 mM.

35. The method of claim 33, wherein additional albumin is added to arrive at a final albumin concentration of 0.39 mM.

36. The method of claim 33, wherein the laminin is an effective recombinant laminin.

37. The method of claim 33, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

38. The method of claim 33, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

39. The method of claim 33, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

40. The method of claim 33, wherein the stem cells are plated at a density of 200 cells / mm^2 or less.

41. The method of claim 33, wherein the stem cells are plated at a density of 200 cells / mm^2 or more.

42. The method of claim 33, wherein the stem cells are plated such that no two stem cells contact each other.