[0040] The composition may be devoid of any feeder cells, such as mouse fibroblasts or human foreskin fibroblasts. The composition may be devoid of any differentiation inductors, such as Noggin or keratinocyte growth factor. In some embodiments, the composition is devoid of any differentiation inhibitors, feeder cells, or differentiation inductors.

[0041] The pluripotent stem cells may be placed on the laminin-521 coating as a monolayer.

[0042] The pluripotent stem cells may be placed on the coating at a density of 200 cells / mm^2 or less. Alternatively, the pluripotent stem cells may be placed on the coating at a density of 200 cells / mm^2 or more. The pluripotent stem cells can alternatively be placed on the coating such that no two stem cells contact each other.

[0043] Also disclosed in embodiments is isolated recombinant laminin-521 produced by a method comprising: providing host cells that express recombinant laminin-521, wherein the recombinant laminin-521 comprises: a first chain comprising a polypeptide with at least 80% identity to a polypeptide sequence of SEQ ID NO: 1, a second chain comprising a polypeptide with at least 70% identity to a polypeptide sequence of SEQ ID NO: 2, and a third chain comprising a polypeptide with at least 70% identity to a polypeptide sequence of SEQ ID NO: 3, wherein the first, second, and third chains are assembled into recombinant laminin-521; growing the host cells in a cell culture medium under conditions to stimulate expression of the recombinant laminin-521 chains; passing the host cell culture medium through a column, wherein the column contains a compound that binds to the recombinant laminin-521; washing the column to remove unbound materials; and eluting the bound recombinant laminin-521 from the column.

[0043a] In one aspect, there is provided 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; and exposing the stem cells to a cell culture medium comprising at least 0.3 mM of albumin; wherein the laminin is either laminin-521 or laminin-511; ~~and~~ wherein the cadherin is e-cadherin; and wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

[0043b] In another aspect, there is provided 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; and exposing the stem cells to a mTeSR1 cell culture medium; wherein the laminin is either laminin-521 or laminin-511; ~~and~~ wherein the cadherin is e-cadherin; and wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

[0044] Described in other embodiments are methods of maintaining the pluripotency of pluripotent stem cells in vitro, comprising: receiving a substrate having a coating thereon, the coating containing an intact laminin; placing pluripotent stem cells and a cell culture medium on the substrate; and activating the PI3-kinase/Akt pathway.

[0045] The intact laminin may be laminin-521 or laminin-511. In some embodiments, the cell culture medium does not contain any growth factors, such as beta fibroblast growth factor (bFGF). The coating may also contain a cadherin. The pluripotent stem cells may be placed on the coating at a density of 200 cells / mm2 or less.

[0046] The present disclosure also relates to cell culture media that can be used to maintain stem cells in a pluripotent state. Described in various embodiments is a cell culture medium that provides nutrition to pluripotent stem cells, comprising from greater than zero to 3.9 ng/mL of basic fibroblast growth factor (bFGF).

[0047] The cell culture medium may comprise 3.5 ng/mL or less of bFGF, including from 0.5 to 3.5 ng/mL of bFGF.

[0048] The cell culture medium may further comprise at least one inorganic salt, at least one trace mineral, at least one energy substrate, at least one lipid, at least one amino acid, at least one vitamin, or at least one additional growth factor. In particular embodiments, the cell culture medium further comprises at least one inorganic salt, at least one trace mineral, at least one energy substrate, at least one lipid, at least one amino acid, and at least one vitamin.

[0049] The cell culture medium may not contain any one of (1) albumin, (2) insulin or an insulin substitute, or (3) transferrin or a transferrin substitute.

[0050] The cell culture medium may further comprise albumin, insulin, lithium chloride, GABA, TGF beta 1, pipecolic acid, L-glutamine, MEM non-essential amino acid solution, and DMEM/F12 solution.

[0051] The cell culture medium may further comprise at least one additional growth factor, at least one trace mineral, and at least one lipid.

[0051a] In another aspect, there is provided 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 the laminin is either laminin-521 or laminin-511; ~~and~~ wherein the cadherin is e-cadherin; and wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

[0051b] In another aspect, there is provided 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 the laminin is either laminin-521 or laminin-511; ~~and~~ wherein the cadherin is e-cadherin; and wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

[0052] Also disclosed is a system for maintaining pluripotent stem cells, comprising: a cell culture medium comprising from greater than zero to 3.9 ng/mL of basic fibroblast growth factor (bFGF); and a substrate for providing support to the stem cells.

[0053] The substrate may contain laminin-521 or laminin-511. The substrate may also contain a cadherin.

[0053a] In one aspect, there is provided a cell culture substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment; wherein the laminin is either laminin-521 or laminin-511; ~~and~~ wherein the cadherin is e-cadherin; and wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

CLAIMS:

1. A cell culture substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment;

wherein the laminin is either laminin-521 or laminin-511;

wherein the cadherin is e-cadherin; and

wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

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

3. The substrate of claim 1, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

4. The substrate of claim 1, wherein the weight ratio of laminin-521 to e-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 the laminin is either laminin-521 or laminin-511;

wherein the cadherin is e-cadherin; and

wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

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 weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

11. The system of claim 5, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

12. The system of claim 5, wherein the weight ratio of laminin-521 to e-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. The system of claim 5, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

15. 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 the laminin is either laminin-521 or laminin-511;

wherein the cadherin is e-cadherin; and

wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

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

17. The system of claim 15, wherein additional albumin is added to arrive at a final albumin concentration of 0.39 mM.

18. The system of claim 15, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

19. The system of claim 15, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

20. The system of claim 15, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

21. The system of claim 15, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

22. The system of claim 15, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the mTeSR1 medium contains 0.39 mM albumin.

23. 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; and

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

wherein the laminin is either laminin-521 or laminin-511;

wherein the cadherin is e-cadherin; and

wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

24. The method of claim 23, wherein the cell culture medium is mTeSR1 medium with additional albumin added to arrive at an albumin concentration of at least 0.3 mM.

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

26. The method of claim 23, wherein the albumin is human serum albumin.

27. The method of claim 26, wherein the human serum albumin is recombinant human serum albumin.

28. The method of claim 23, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

29. The method of claim 23, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

30. The method of claim 23, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

31. The method of claim 23, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

32. The method of claim 23, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

33. The method of claim 23, wherein the stem cells are plated at a density of 200 cells / mm^2 or less.

34. The method of claim 23, wherein the stem cells are plated at a density of 200 cells / mm^2 or more.

35. The method of claim 23, wherein the stem cells are plated such that no two stem cells contact each other.

36. 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; and

exposing the stem cells to a mTeSR1 cell culture medium;

wherein the laminin is either laminin-521 or laminin-511;

wherein the cadherin is e-cadherin; and

wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

37. The method of claim 36, wherein additional albumin is added to arrive at a final albumin concentration of at least 0.3 mM.

38. The method of claim 36, wherein additional albumin is added to arrive at a final albumin concentration of 0.39 mM.

39. The method of claim 36, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

40. The method of claim 36, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

41. The method of claim 36, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

42. The method of claim 36, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

43. The method of claim 36, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the mTeSR1 medium contains 0.39 mM albumin.

44. The method of claim 36, wherein the stem cells are plated at a density of 200 cells / mm^2 or less.

45. The method of claim 36, wherein the stem cells are plated at a density of 200 cells / mm^2 or more.

46. The method of claim 36, wherein the stem cells are plated such that no two stem cells contact each other.

CLAIMS:

1. A cell culture substrate comprising a laminin and a cadherin, wherein the laminin is an intact protein or a protein fragment;

wherein the laminin is either laminin-521 or laminin-511; and

wherein the cadherin is e-cadherin.

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

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

4. The substrate of claim 1, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

5. The substrate of claim 1, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

6. 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 the laminin is either laminin-521 or laminin-511; and

wherein the cadherin is e-cadherin.

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

8. The system of claim 6, wherein the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

9. The system of claim 6, wherein the albumin is human serum albumin.

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

11. The system of claim 6, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

12. The system of claim 6, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

13. The system of claim 6, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

14. The system of claim 6, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

15. The system of claim 6, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

16. The system of claim 6, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

17. 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 the laminin is either laminin-521 or laminin-511; and

wherein the cadherin is e-cadherin.

18. The system of claim 17, wherein additional albumin is added to arrive at a final albumin concentration of at least 0.3 mM.

19. The system of claim 17, wherein additional albumin is added to arrive at a final albumin concentration of 0.39 mM.

20. The system of claim 17, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

21. The system of claim 17, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

22. The system of claim 17, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

23. The system of claim 17, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

24. The system of claim 17, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

25. The system of claim 17, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the mTeSR1 medium contains 0.39 mM albumin.

26. 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; and

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

wherein the laminin is either laminin-521 or laminin-511; and

wherein the cadherin is e-cadherin.

27. The method of claim 26, wherein the cell culture medium is mTeSR1 medium with additional albumin added to arrive at an albumin concentration of at least 0.3 mM.

28. The method of claim 26, wherein the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

29. The method of claim 26, wherein the albumin is human serum albumin.

30. The method of claim 26, wherein the human serum albumin is recombinant human serum albumin.

31. The method of claim 26, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

32. The method of claim 26, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

33. The method of claim 26, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

34. The method of claim 26, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

35. The method of claim 26, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

36. The method of claim 26, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the cell culture medium is mTeSR1 medium with 0.39 mM albumin.

37. The method of claim 26, wherein the stem cells are plated at a density of 200 cells / mm^2 or less.

38. The method of claim 26, wherein the stem cells are plated at a density of 200 cells / mm^2 or more.

39. The method of claim 26, wherein the stem cells are plated such that no two stem cells contact each other.

40. 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; and

exposing the stem cells to a mTeSR1 cell culture medium;

wherein the laminin is either laminin-521 or laminin-511; and

wherein the cadherin is e-cadherin.

41. The method of claim 40, wherein additional albumin is added to arrive at a final albumin concentration of at least 0.3 mM.

42. The method of claim 40, wherein additional albumin is added to arrive at a final albumin concentration of 0.39 mM.

43. The method of claim 40, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 15:1.

44. The method of claim 40, wherein the weight ratio of the laminin to the cadherin is from 5:1 to 10:1.

45. The method of claim 40, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1.

46. The method of claim 40, wherein the weight ratio of laminin-521 to e-cadherin is from 5:1 to 10:1.

47. The method of claim 40, wherein the substrate and the medium do not contain any differentiation inhibitors, feeder cells, differentiation inductors, or apoptosis inhibitors.

48. The method of claim 40, wherein the laminin is laminin-521, the weight ratio of laminin-521 to e-cadherin is from 5:1 to 15:1, and the mTeSR1 medium contains 0.39 mM albumin.

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

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

51. The method of claim 40, wherein the stem cells are plated such that no two stem cells contact each other.