[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.

~~BRIEF DESCRIPTION~~

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 aspects, the present disclosure provides recombinant host cells that express laminin-521 chains and secrete recombinant laminin-521.

[0021] In other ~~aspects~~embodiments, 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 embodiments 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.

[0024] In further ~~aspects~~embodiments, 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,

and replating the stem cells from single cell suspension as single cells on a matrix containing laminin-521 such that this process can be performed in automated robotic systems with high efficiency.

[0025] In further ~~aspects~~embodiments, the present disclosure provides improved medical devices and grafts, wherein the improvement comprises providing medical devices and grafts with an effective amount of isolated laminin-521.

[0026] In further ~~aspects~~embodiments, the disclosure provides improved cell culture devices, and methods for preparing improved cell culture devices, for the growth and maintenance of phenotypes of cells in culture, by providing an effective amount of isolated laminin-521 to a cell culture device for cell attachment, and subsequent cell stasis, proliferation, differentiation, and/or migration.

[0027] In other ~~aspect~~embodiment, the disclosure provides compositions, such as human recombinant laminin-521, and methods for culturing and rapid expanding of human embryonic stem cells in vitro in undifferentiated state. The methods comprise single cell dissociation of human embryonic stem cells and plating them on laminin-521 coated cell culture dishes in medium without any Rho-associated kinase (ROCK) inhibitor (e.g. without Y-27632) (Watanabe et al., *Nature Biotechnology* **25**, 681 - 686 (2007)). The improvement provides a possibility to expand human embryonic stem cells in pluripotent state faster than in conventional human embryonic stem cell cultures.

[0028] In further ~~aspects~~embodiments, the disclosure provides new materials, such as human recombinant laminin-521, which permit human embryonic cell survival after dissociation into single cell suspension. The improvement provides better cloning survival of human embryonic stem cells, which may be advantageous for isolating clones (e.g. after genetic manipulations) or obtaining homogeneous and uniformal human embryonic stem cell populations.

[0029] Disclosed in some embodiments is isolated recombinant laminin-521, comprising: an alpha chain comprising a polypeptide with at least 80% identity to a polypeptide sequence of SEQ ID NO: 1; a beta chain comprising a polypeptide with at least 70% identity to a polypeptide sequence of SEQ ID NO: 2; and a gamma chain comprising a polypeptide with at least 70% identity to a polypeptide sequence of SEQ ID NO: 3; wherein the alpha, beta, and gamma chains are assembled into recombinant laminin-521.

[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.

[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.

[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.

[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.

[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.

[0054] These and other non-limiting characteristics of the disclosure are more particularly ~~disclosed~~ described below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0055] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.