[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

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

[0021] In other embodimentaspects, 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 embodimentaspects, 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 embodiment~~aspect~~s, 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.

[0025a] In one aspect, there is provided a medical device or a graft having an exterior surface, the exterior surface being coated with isolated recombinant laminin-521.

[0026] In further embodiment~~aspect~~s, 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 embodiment~~aspect~~, 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 embodiment~~aspect~~s, 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.

[0030] In further embodiments, the alpha chain polypeptide has at least 90% identity to the polypeptide sequence of SEQ ID NO: 1. The beta chain polypeptide may also have at least 90% identity to the polypeptide sequence of SEQ ID NO: 2. The gamma chain polypeptide may also have at least 90% identity to the polypeptide sequence of SEQ ID NO: 3.

[0031] In other embodiments, the alpha chain has the polypeptide sequence of SEQ ID NO: 1. Furthermore, the beta chain may have the polypeptide sequence of SEQ ID NO: 2. Even more specifically, the gamma chain may have the polypeptide sequence of SEQ ID NO: 3.

[0032] The beta chain polypeptide may have at least 80% identity to the polypeptide sequence of SEQ ID NO: 2. The gamma chain polypeptide may have at least 80% identity to the polypeptide sequence of SEQ ID NO: 3.

[0033] Also disclosed in embodiments is a pharmaceutical composition, comprising: a) the isolated recombinant laminin-521 described above; and b) a pharmaceutically acceptable carrier.

[0034] In one aspect, there is provided~~Also disclosed in embodiments is~~ a composition that enables self-renewal of pluripotent stem cells grown *in vitro*, comprising a growth medium and a coating thereover, the coating comprising recombinant laminin 521 (laminin-11).

[0035] *The composition may further comprise a growth factor.*

[0036] The composition may be devoid of any differentiation inhibitors. The composition may also be devoid of any feeder cells. The composition may also be devoid of any differentiation inductors. In some embodiments, the composition is devoid of any differentiation inhibitors, feeder cells, or differentiation inductors.

[0037] In one aspect, there is provided~~Also disclosed in embodiments is~~ a method for maintaining the pluripotency of pluripotent stem cells *in vitro*, comprising: providing a substrate comprising a growth medium and a coating thereover, the coating comprising a matrix of recombinant laminin 521 (laminin-11)~~ such as that described above~~; dissociating pluripotent stem cells into a single cell suspension; and placing the pluripotent stem cells in the single cell suspension on the coating.

[0038] The growth medium may comprise a growth factor or growth factors. Exemplary growth factors include basic fibroblast growth factor and insulin growth factor.

[0039] Sometimes, the growth medium and the coating are devoid of any differentiation inhibitors. For example, leukemia inhibitor factor is not present.

[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.

[0044] In one aspect, there is provided a~~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.