[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

[0019a] In one aspect, there is provided a system for maintaining pluripotent stem cells, comprising:

a cell culture medium comprising from 0.5 to 3.5 ng/mL of basic fibroblast growth factor (bFGF); and

a substrate containing laminin-521 for providing support to the stem cells.

[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~~embodiments, 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.