culture to animal pathogens and immunogenic agents. Moreover, fetal bovine serum (FBS) used in the culture media is undefined and has batch-to-batch variation.

[0005] To overcome this problem, serum-free and xeno-free cell culture media have been developed for keratinocyte cultures. However, these systems remain non-optimal and none are used in clinical applications for severe wound defects. It has been demonstrated that culturing keratinocytes using serum-free keratinocyte growth media without the use of irradiated 3T3 feeder cells could not preserve the proliferative capacity of keratinocytes compared to those supported by feeder cells.

[0006] It would be desirable to develop methods for supporting keratinocyte survival and expansion *in vitro* without the aid of 3T3 feeder cells and animal-derived serum.

BRIEF DESCRIPTION

[0007] The present disclosure provides methods for culturing human epidermal keratinocytes. These keratinocytes can then be used in therapies such as treating and managing less severe burns and chronic wounds. More particularly, this disclosure also describes a novel system for culturing human epidermal keratinocytes in a completely xeno-free and fully human method. By using pure laminin matrices (for example, LN-511 and/or LN-421) and chemically defined, serum-free medium, this method supports keratinocyte survival *in vitro* without the aid of feeder cells and xenobiotic components, with as many as 30 population doublings in adult patient cells. In further examples, the present disclosure provides a kit for culturing keratinocytes.

[0008] FACS analysis and immunofluorescence staining confirm that cells grown in this system express keratinocytes basal markers KRT5, KRT14, KRT15, ITGA6, and ITGB1. Differentiation markers KRT1 and KRT10 are expressed normally at the later passage. Real-time Quantitative PCR analyses (qPCR) carried out at different time points show the keratinocyte basal and differentiation profile over increasing passages.

[0009] In some examples, the disclosure describes the plating of keratinocytes on a substrate of Laminin-411 (LN-411), Laminin-421 (LN-421), Laminin-511 (LN-511), or Laminin-521 (LN-521), and culturing the cells using a cell culture medium. In other words, the laminin used in the substrate contains an alpha-4 or alpha-5 chain. In

particular examples, the cell culture medium is xeno-free. The cells may be passaged with Trypsin EDTA prior to plating.

[0010] In particular examples the cell culture medium is a basal medium. According to some examples, the basal medium includes insulin and chemically defined growth supplement. Other ingredients can include human epidermal growth factor (hEGF); hydrocortisone; and antibiotics and/or antifungals such as gentamicin or amphotericin B. The insulin may be recombinant human insulin. In additional examples, the basal medium further includes epinephrine and transferrin.

[0011] In some examples, freshly isolated keratinocytes may be plated on the substrate at a density of about $1x10^5/cm^2$. In specific examples, keratinocytes are cultured in an atmosphere containing from about 5% CO_2 to about 15% CO_2. The keratinocytes may be cultured at a temperature from about 30 degrees Celsius to about 40 degrees Celsius.

[0012] In other examples, the keratinocytes may be trypsinized at a temperature from about 30 degrees Celsius to about 40 degrees Celsius. In particular examples, keratinocytes are trypsinized for a period of about 4 minutes to about 12 minutes. In more specific examples, keratinocytes are trypsinized for a period of about 6 minutes to about 10 minutes. In further particular examples, keratinocytes are passaged upon reaching a density of about $1x10^4$ cells/cm^2.

[0012a] In one aspect, the present disclosure refers to a method for expanding keratinocytes, comprising:

plating keratinocytes in a completely chemically defined environment and xeno-free conditions using a system comprising:

a substrate comprising recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521; and

a xeno-free cell culture medium.

[0013] Also disclosed is a system for maintaining keratinocytes, comprising a substrate containing Laminin-411, Laminin-421, Laminin-511, and/or Laminin-521 and a cell culture medium. In particular examples, the cell culture medium is xeno-free. In other examples, the cell culture medium does not contain animal serum.

[0014] The present disclosure also describes a kit for culturing keratinocytes in vitro. The kit includes: a cell culture plate with a coating of Laminin-411, Laminin-421, Laminin-511, and/or Laminin-521; and a cell culture medium, wherein the cell culture medium is serum-free.

[0015] In one aspect, the present disclosure refers to use of keratinocyte cells in the manufacture of a medicament for treating a burn wound of a patient, wherein the use comprises: plating keratinocyte cells taken from a donor site of the patient on a substrate coated with recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521; expanding the keratinocyte cells on the substrate in a completely chemically defined environment and xeno-free conditions; and covering the burn wound with the keratinocyte cells from the substrate. Also disclosed herein are methods for treating a burn wound of a patient. Keratinocyte cells taken from a donor site of the patient are plated on a substrate coated with Laminin-411, Laminin-421, Laminin-511, and/or Laminin-521. The

keratinocyte cells are expanded on the substrate. The keratinocyte cells from the substrate are then used to cover the burn wound.

[0016] In one aspect, the present disclosure refers to use of keratinocyte cells in the manufacture of a medicament for treating a chronic wound of a patient, wherein the use comprises: plating keratinocyte cells taken from a donor site of the patient on a substrate coated with recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521; expanding the keratinocyte cells on the substrate in a completely chemically defined environment and xeno-free conditions; and covering the wound with the keratinocyte cells from the substrate. Also disclosed herein are methods for treating a chronic wound of a patient. Keratinocyte cells taken from a donor site of the patient are plated on a substrate coated with Laminin-411, Laminin-421, Laminin-511, and/or Laminin-521. The keratinocyte cells are expanded on the substrate. The keratinocyte cells from the substrate are then used to cover the wound.

[0017] In one aspect, the present disclosure refers to use of keratinocyte cells in the manufacture of a medicament for treating any injuries or conditions in the human body where the epithelium is compromised, and/or where an epithelium defect is a component of the injury, wherein the use comprises: plating keratinocyte cells taken from a donor site of the patient on a substrate coated with recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521; expanding the keratinocyte cells on the substrate in a completely chemically defined environment and xeno-free conditions; and covering the epithelium defect with the keratinocyte cells from the substrate. Also disclosed herein are methods for treating any injuries or conditions in the human body, including cases in which only the epithelium is compromised, and/or in which an epithelium defect is a component of the injury. Keratinocyte cells taken from a donor site of the patient are plated on a substrate coated with Laminin-411, Laminin-421, Laminin-511, and/or Laminin-521. The keratinocyte cells are expanded on the substrate. The keratinocyte cells from the substrate are then used to cover the epithelium defect.

[0018] Also disclosed herein is the use of keratinocytes for the treatment of burn wounds, chronic wounds, and other epithelium defects. Desirably, the keratinocytes were plated and expanded on a substrate coated with Laminin-411, Laminin-421, Laminin-511, and/or Laminin-521.

[0019] These and other non-limiting characteristics of the disclosure are more particularly disclosed below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] 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.

[0021] The following is a brief description of the drawings, which are presented for the purposes of illustrating the exemplary embodiments disclosed herein and not for the purposes of limiting the same.

[0022] **FIGS. 1A-1J** are a set of images of freshly isolated human epidermal keratinocytes cultured on various laminin matrices and compared to a negative control substrate containing no laminin and a positive control substrate where the keratinocytes

CLAIMS:

1. A method for expanding keratinocytes, comprising:

plating keratinocytes in a completely chemically defined environment and xeno-free conditions using a system comprising:

a substrate comprising recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521; and

a xeno-free cell culture medium.

2. The method of claim 1, wherein the keratinocytes are plated on the substrate at a density of about $0.7x10^5/cm^2$ to about $1.2x10^5/cm^2$.

3. The method of claim 1, wherein the keratinocytes are cultured on the substrate at a temperature from about 30 degrees Celsius to about 40 degrees Celsius.

4. The method of claim 1, wherein the keratinocytes are cultured in an atmosphere containing from about 5% CO_2 to about 15% CO_2.

5. The method of claim 1, further comprising periodically dissociating and passaging the keratinocytes.

6. The method of claim 5, wherein the passaging of the keratinocytes occurs upon reaching a density of 1 cell to about $1.5x10^4$ cells/cm^2.

7. The method of claim 1, wherein the substrate is coated with both recombinant laminin-511 and recombinant laminin-421.

8. The method of claim 7, wherein the weight ratio of laminin-511 to laminin-421 is from 15:1 to 1:15.

9. Use of keratinocyte cells in the manufacture of a medicament for treating a burn wound of a patient, wherein the use comprises:

plating keratinocyte cells taken from a donor site of the patient on a substrate coated with recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521;

expanding the keratinocyte cells on the substrate in a completely chemically defined environment and xeno-free conditions; and

covering the burn wound with the keratinocyte cells from the substrate.

10. Use of keratinocyte cells in the manufacture of a medicament for treating a chronic wound of a patient, wherein the use comprises:

plating keratinocyte cells taken from a donor site of the patient on a substrate coated with recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521;

expanding the keratinocyte cells on the substrate in a completely chemically defined environment and xeno-free conditions; and

covering the wound with the keratinocyte cells from the substrate.

11. Use of keratinocyte cells in the manufacture of a medicament for treating any injuries or conditions in the human body where the epithelium is compromised, and/or where an epithelium defect is a component of the injury, wherein the use comprises:

plating keratinocyte cells taken from a donor site of the patient on a substrate coated with recombinant Laminin-411, Laminin-421, Laminin-511, or Laminin-521;

expanding the keratinocyte cells on the substrate in a completely chemically defined environment and xeno-free conditions; and

covering the epithelium defect with the keratinocyte cells from the substrate.

12. The use of claim 11, wherein the injuries or conditions in the human body are in an eye, the bladder, the oral cavity, or the intestine.