variations, and contains an extensive amount of undefined components. Moreover, cells cultured on Matrigel™ also have the possibility of acquiring non-human N-glycolylneuraminic acid (Neu5Gc) immunogen which renders them unsuitable for clinical applications.

[0006] Current treatments include oral medications that include antioxidants (e.g. vitamin A palmitate), docosahexaenoic acid (DHA), calcium channel blockers, and lutein/zeaxanthin. However, none show effectiveness in restoring vision.

SUMMARY

[0007] The present disclosure relates to systems and methods for obtaining retinal progenitor cells or retinal photoreceptor cells. Briefly, pluripotent stem cells are cultured on a substrate comprising a mixture of (i) laminin-323 (LN-323) or LN-523, and (ii) laminin-521. LN-323 and LN-523 have not previously been purified in pure form, and were produced for the first time as recombinant human proteins in human embryonic kidney cells (HEK-293), purified and as cell culture coatings. The stem cells are differentiated using two different cell culture mediums to obtain the retinal progenitor cells or retinal photoreceptor cells (depending on the length of time they are differentiated).

[0008] Five distinct subpopulations of retinal progenitor cells have also been identified. These five subpopulations can be identified by a Day 26-34 gene profile, as explained further herein.

[0009] In the first subpopulation (A), at least one of genes *CRX*, *C11orf96*, *NXPH4*, *NTS*, *DCT*, *PRDM1*, *NEUROD4*, *S100A13*, *RCVRN*, *FAM57B*, *SYT4*, *DLL3*, *SSTR2*, *CHRNA5*, and *ROBO2* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

[0010] In the second subpopulation (B), at least one of genes *STMN2*, *ONECUT2*, *ATOH7*, *ELAVL4*, and *GAP43* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

[0011] In the third subpopulation (C), at least one of genes *CNTN2*, *NEFM*, *NEFL*, *PRPH*, *POU4F2*, and *CXCR4* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

[0012] In the fourth subpopulation (D), at least one of genes *PAX6*, *SIX3*, and *SLC2A1* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

[0013] In the fifth subpopulation (E), at least one of genes *VSX2*, *PTH2*, *FZD5*, *RARRES2*, *DIO3*, *SOX2*, and *CYP1B1* is expressed in the Day 26-34 gene profile. Desirably, more or most of these genes are expressed.

[0013a] In one aspect, the present disclosure refers to a photoreceptor progenitor cell, wherein a Day 26-34 gene profile indicates that:

(A) at least one of genes CRX, C11orf96, NXPH4, NTS, DCT, PRDM1, NEUROD4, S100A13, RCVRN, FAM57B, SYT4, DLL3, SSTR2, CHRNA5, and ROBO2 is expressed; or

(B) at least one of genes STMN2, ONECUT2, ATOH7, ELAVL4, and GAP43 is expressed; or

(C) at least one of genes CNTN2, NEFM, NEFL, PRPH, POU4F2, and CXCR4 is expressed; or

(D) at least one of genes PAX6, SIX3, and SLC2A1 is expressed; or

(E) at least one of genes VSX2, PTH2, FZD5, RARRES2, DIO3, SOX2, and CYP1B1 is expressed.

[0013b] In one aspect, the present disclosure refers to a method for producing a population of photoreceptor progenitors, comprising:

plating pluripotent human stem cells on a cell culture surface having a laminin matrix thereon, wherein the laminin matrix comprises (i) a first laminin which is laminin-521, wherein the laminin-521 is either an intact protein or a protein fragment;

culturing the pluripotent human stem cells to obtain photoreceptor progenitors;

identifying the population of photoreceptor progenitors by (i) preparing a Day 16-24 gene profile and selecting photoreceptor progenitors in which at least one of genes DAPL1, SIX6 SFRP2, LHX2, *PAX6*, CDH2, CLDN1, TRPM3, RELN, DCT and PMEL is expressed; and (ii) preparing a Day 26-34 gene profile and selecting photoreceptor progenitors in which:

(A) at least one of genes *CRX*, C11orf96, NXPH4, NTS, DCT, PRDM1, NEUROD4, S100A13, *RCVRN*, FAM57B, SYT4, DLL3, SSTR2, CHRNA5, and ROBO2 are expressed; or

(B) at least one of genes STMN2, ONECUT2, ATOH7, ELAVL4, and GAP43 are expressed; or

(C) at least one of genes CNTN2, NEFM, NEFL, PRPH, POU4F2, and CXCR4 are expressed; or

(D) at least one of genes PAX6, SIX3, and SLC2A1 are expressed; or

(E) at least one of genes VSX2, PTH2, FZD5, RARRES2, DIO3, SOX2, and CYP1B1 are expressed.

[0013c] In one aspect, the present disclosure refers to a method for producing recombinant laminin-323 or recombinant laminin-523, comprising:

encoding a first expression plasmid with (A) a sequence that codes for laminin alpha-3 or alpha-5 chain, and (B) a first antibiotic resistance gene;

encoding a second expression plasmid with (A) a sequence that codes for laminin beta-2 chain, and (B) a second antibiotic resistance gene;

encoding a third expression plasmid with (A) a sequence that codes for laminin gamma-3 chain, and (B) a third antibiotic resistance gene;

transfecting the first, second, and third expression plasmids into human cells that are maintained in a basal medium containing serum;

identifying potential positive clones;

exposing the potential positive clones to a serum-free cell culture medium;

filtering the serum-free cell culture medium to obtain the recombinant laminin-323 or recombinant laminin-523;

optionally concentrating the recombinant laminin-323 or recombinant laminin-523 by centrifugation after filtering; and

optionally purifying the recombinant laminin-323 or recombinant laminin-523.

[0014] These and other non-limiting characteristics of the disclosure are more particularly disclosed below.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] 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.

[0016] 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.

[0017] **FIG. 1A** is a flow chart describing the steps for producing recombinant laminin-523 and laminin-323 in some methods of the present disclosure.

[0018] **FIG. 1B** is a Western blot showing the presence of LAMA5, LAMB2, and LAMC3 chains in the purified fractions or parts of LN-523.

[0019] **FIG. 1C** is a chromatogram illustrating the protein elution profile of a LN-523 clone using the AKTA Chromatography System.

[0020] **FIG. 1D** is LC-MS/MS (Liquid Chromatography-Mass Spectrophotometry) results showing that LAMA5, LAMB2, and LAMC3 chains are present.

[0021] **FIG. 1E** is a Western blot showing the presence of LAMA3, LAMB2, and LAMC3 chains in the purified fractions or parts of LN-323.

[0022] **FIG. 1F** is a chromatogram illustrating the protein elution profile of a LN-323 clone using the AKTA Chromatography System.

[0023] **FIG. 1G** is LC-MS/MS (Liquid Chromatography-Mass Spectrophotometry) results showing that LAMA3, LAMB2, and LAMC3 chains are present.

[0024] **FIG. 2A** is a diagram depicting a method of differentiating hESCs to photoreceptors. Three cell cultures are indicated: Nutristem for culturing prior to

CLAIMS:

1. A photoreceptor progenitor cell, wherein a Day 26-34 gene profile indicates that:

(A) at least one of genes *CRX*, *C11orf96*, *NXPH4*, *NTS*, *DCT*, *PRDM1*, *NEUROD4*, *S100A13*, *RCVRN*, *FAM57B*, *SYT4*, *DLL3*, *SSTR2*, *CHRNA5*, and *ROBO2* is expressed; or

(B) at least one of genes *STMN2*, *ONECUT2*, *ATOH7*, *ELAVL4*, and *GAP43* is expressed; or

(C) at least one of genes *CNTN2*, *NEFM*, *NEFL*, *PRPH*, *POU4F2*, and *CXCR4* is expressed; or

(D) at least one of genes *PAX6*, *SIX3*, and *SLC2A1* is expressed; or

(E) at least one of genes *VSX2*, *PTH2*, *FZD5*, *RARRES2*, *DIO3*, *SOX2*, and *CYP1B1* is expressed.

2. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that:

(A) at least two of genes *CRX*, *C11orf96*, *NXPH4*, *NTS*, *DCT*, *PRDM1*, *NEUROD4*, *S100A13*, *RCVRN*, *FAM57B*, *SYT4*, *DLL3*, *SSTR2*, *CHRNA5*, and *ROBO2* are expressed; or

(B) at least two of genes *STMN2*, *ONECUT2*, *ATOH7*, *ELAVL4*, and *GAP43* are expressed; or

(C) at least two of genes *CNTN2*, *NEFM*, *NEFL*, *PRPH*, *POU4F2*, and *CXCR4* are expressed; or

(D) at least two of genes *PAX6*, *SIX3*, and *SLC2A1* are expressed; or

(E) at least two of genes *VSX2*, *PTH2*, *FZD5*, *RARRES2*, *DIO3*, *SOX2*, and *CYP1B1* are expressed.

3. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that gene *CRX* or *C11orf96* is expressed; and

wherein the Day 26-34 gene profile indicates that (i) *CRX* is expressed with at least 5 gene count-per-million, or (ii) *C11orf96* is expressed with at least 5 gene count-per-million.

4. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that gene *STMN2* or *ONECUT2* is expressed; and

wherein the Day 26-34 gene profile indicates that (i) *STMN2* is expressed with at least 5 gene count-per-million, or (ii) *ONECUT2* is expressed with at least 5 gene count-per-million.

5. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that gene *CNTN2* or *POU4F2* is expressed; and

wherein the Day 26-34 gene profile indicates that (i) *CNTN2* is expressed with at least 5 gene count-per-million, or (ii) *POU4F2* is expressed with at least 5 gene count-per-million.

6. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that gene *PAX6* or *SIX3* is expressed; and

wherein the Day 26-34 gene profile indicates that (i) *PAX6* is expressed with at least 5 gene count-per-million, or (ii) *SIX3* is expressed with at least 5 gene count-per-million.

7. The photoreceptor progenitor cell of claim 1, wherein the Day 26-34 gene profile indicates that gene *VSX2* or *CYP1B1* is expressed; and

wherein the Day 26-34 profile indicates that (i) *VSX2* is expressed with at least 5 gene count-per-million, or (ii) *CYP1B1* is expressed with at least 5 gene count-per-million.

8. The photoreceptor progenitor cell of claim 1, wherein a Day 16-24 gene profile indicates that at least one of genes *DAPL1*, *SIX6*, *SFRP2*, *LHX2*, *PAX6*, *CDH2*, *CLDN1*, *TRPM3*, *RELN*, *DCT*, and *PMEL* is expressed.

9. The photoreceptor progenitor cell of claim 8, wherein the Day 16-24 gene profile indicates that *PAX6* is expressed.

10. A method for producing a population of photoreceptor progenitors, comprising:

plating pluripotent human stem cells on a cell culture surface having a laminin matrix thereon, wherein the laminin matrix comprises (i) a first laminin which is laminin-521, wherein the laminin-521 is either an intact protein or a protein fragment;

culturing the pluripotent human stem cells to obtain photoreceptor progenitors;

identifying the population of photoreceptor progenitors by (i) preparing a Day 16-24 gene profile and selecting photoreceptor progenitors in which at least one of genes DAPL1, SIX6 SFRP2, LHX2, *PAX6*, CDH2, CLDN1, TRPM3, RELN, DCT and PMEL is expressed; and (ii) preparing a Day 26-34 gene profile and selecting photoreceptor progenitors in which:

(A) at least one of genes *CRX*, C11orf96, NXPH4, NTS, DCT, PRDM1, NEUROD4, S100A13, *RCVRN*, FAM57B, SYT4, DLL3, SSTR2, CHRNA5, and ROBO2 are expressed; or

(B) at least one of genes STMN2, ONECUT2, ATOH7, ELAVL4, and GAP43 are expressed; or

(C) at least one of genes CNTN2, NEFM, NEFL, PRPH, POU4F2, and CXCR4 are expressed; or

(D) at least one of genes *PAX6*, SIX3, and SLC2A1 are expressed; or

(E) at least one of genes *VSX2*, PTH2, FZD5, RARRES2, DIO3, SOX2, and CYP1B1 are expressed.

11. The method of claim 10, wherein the pluripotent human stem cells are cultured by:

culturing the cells in a neural induction medium comprising a TGFβ inhibitor and a Wnt inhibitor for a first time period; and

culturing the cells in a photoreceptor differentiation medium comprising a brain derived neurotrophic factor (BDNF), a ciliary neutrotrophic factor (CNTF), retinoic acid, and a gamma secretase inhibitor for a second time period.

12. The method of claim 11, wherein the first time period is from about 120 hours to about 168 hours.

13. The method of claim 11, wherein the second time period is from about 288 hours to about 816 hours.

14. The method of claim 11, wherein the neural induction medium comprises:
from about 5 µM to about 15 µM of the TGFβ inhibitor; and
from about 5 µM to about 15 µM of the Wnt inhibitor.

15. The method of claim 11, wherein:
the TGFβ inhibitor is SB431542; or
the Wnt inhibitor is CKI-7.

16. The method of claim 11, wherein the photoreceptor differentiation medium comprises:
from about 1 ng/mL to about 20 ng/mL of the brain derived neurotrophic factor (BDNF);
from about 1 ng/mL to about 30 ng/mL of the ciliary neutrotrophic factor (CNTF);
from about 0.1 µM to about 5 µM of the retinoic acid; and
from about 5 µM to about 15 µM of the gamma secretase inhibitor.

17. The method of claim 11, wherein the gamma secretase inhibitor is DAPT.

18. The method of claim 10, wherein the laminin matrix further comprises (ii) a second laminin selected from the group consisting of laminin-323 and laminin-523, the second laminin is either an intact protein or a protein fragment;

19. The method of claim 18, wherein the weight ratio of the second laminin to the first laminin is from about 1:1 to about 4:1.

20. A method for producing recombinant laminin-323 or recombinant laminin-523, comprising:

encoding a first expression plasmid with (A) a sequence that codes for laminin alpha-3 or alpha-5 chain, and (B) a first antibiotic resistance gene;

encoding a second expression plasmid with (A) a sequence that codes for laminin beta-2 chain, and (B) a second antibiotic resistance gene;

encoding a third expression plasmid with (A) a sequence that codes for laminin gamma-3 chain, and (B) a third antibiotic resistance gene;

transfecting the first, second, and third expression plasmids into human cells that are maintained in a basal medium containing serum;

identifying potential positive clones;

exposing the potential positive clones to a serum-free cell culture medium;

filtering the serum-free cell culture medium to obtain the recombinant laminin-323 or recombinant laminin-523;

optionally concentrating the recombinant laminin-323 or recombinant laminin-523 by centrifugation after filtering; and

optionally purifying the recombinant laminin-323 or recombinant laminin-523.