SUMMARY

[0006] Provided herein is a method of obtaining a three-dimensional multilayered skin composition. As described herein, the method comprises (a) culturing human pluripotent stem cell aggregates in a culture medium comprising Bone Morphogenetic Protein 4 (BMP4) and an inhibitor of Transforming Growth Factor Beta (TGFβ) signaling for about eight to about ten days, whereby non-neural epithelium (also known as surface ectoderm) forms within the aggregates; (b) embedding the cultured aggregates of (a) in a semi-solid culture medium comprising extracellular matrix; and (c) culturing the embedded aggregates of (b) for about 25 to about 30 days under conditions that promote self-assembly of cells within the embedded aggregates into three-dimensional, multilayered composition comprising an epidermal layer, a dermal layer, and a plurality of cells capable of forming a functional hair follicle is obtained. In a first aspect, there is provided a method of obtaining a three-dimensional multilayered human skin composition, the method comprising: (a) culturing human pluripotent stem cell aggregates in floating culture in a culture medium comprising FGF-2 and SB-43154 for about four days, whereby non-neural epithelium forms within the aggregates; (b) culturing the cultured aggregates of (a) in a culture medium comprising an FGF and LDN- for eight days; (c) embedding the cultured aggregates of (b) in a porous substrate comprising extracellular matrix components; and (d) culturing the embedded aggregates of (c) in floating culture for at least 48 days under conditions that promote self-assembly of cells within the embedded aggregates into the three-dimensional, multilayered human skin composition comprising an epidermal layer, a dermal layer, and a plurality of cells forming a functional hair follicle. The inhibitor of TGFβ1-mediated signaling can be selected from the group consisting of SB431542 and A-83-01. The extracellular matrix can be a basement membrane extract (BME). The epidermal layer can be in direct contact with the dermal layer. The epidermal layer can comprise $P63^{+}KRT5^{+}$ epidermal keratinocytes. The dermal layer comprises follicle-initiating dermal papilla cells. The plurality of cells capable of forming a functional hair follicle can comprise cells selected from the group consisting of mesenchymal stem cells, dermal papilla cells, dermal sheath cells, and follicular epidermal stem cells.

[0007] In one example, the three-dimensional, multilayered engineered skin composition comprises an epidermal layer comprising *in vitro*-derived epidermal keratinocytes, a dermal layer comprising *in vitro*-derived dermal fibroblasts, and a plurality of cells capable of forming a functional hair follicle, wherein the epidermal layer and dermal layer are in direct contact. In a second aspect, there is provided a three-dimensional, multilayered engineered human skin composition comprising an epidermal layer comprising human pluripotent stem cell-derived epidermal keratinocytes, a dermal layer comprising human pluripotent stem cell-derived dermal fibroblasts, a plurality of human pluripotent stem cell-derived cells forming a functional hair follicle, wherein the epidermal layer and dermal layer are in direct contact. The plurality of cells capable of forming a functional hair follicle can comprise cells selected from the group consisting of mesenchymal stem cells, dermal papilla cells, dermal sheath cells, and follicular epidermal stem cells. The composition can further comprise at least one functional hair follicle and at least one functional sebaceous gland. The composition can further comprise a scaffold. The scaffold can be biodegradable or bioabsorbable. The composition can be capable of viable transplantation and engraftment into a human subject.

[0008] Provided herein is a method testing a compound for effects on hair growth or development, the method comprising exposing a test compound to the engineered skin composition as provided herein and detecting a change in hair growth or development.

[0008a] In a third aspect, there is provided a method of testing a compound for effects on hair growth, the method comprising (a) contacting a test compound to a three-dimensional multilayered human skin composition obtained according to the method of the first aspect or to the three-dimensional, multilayered engineered human skin composition as described herein; and (b) detecting an effect of the agent on one or more cell types within the contacted skin composition.

[0008b] In a fourth aspect, there is provided use of a three-dimensional multilayered human skin composition obtained according to the method of the first aspect or the three-dimensional, multilayered engineered human skin composition as described herein in a drug discovery screen.

[0009] These and other features, aspects, and advantages described herein will become better understood upon consideration of the following drawings, detailed description, and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[00010] FIG. 1 is a schematic illustrating an exemplary protocol for deriving human skin organoids from human pluripotent stem cells (hPSCs). hPSC aggregates are cultured in a chemically-defined medium in the presence of extracellular matrix (ECM) components to produce a non-neural ectoderm (also known as surface ectoderm) epithelium. The surface ectoderm epithelium gives rise to both surface epithelial cells and cranial neural crest-like cells (CNCCs). In the second phase of culture, while floating in a chemically defined organoid medium, the CNCCs and epithelia self-organize into cysts having protruding hair follicles. Recommended culture formats are denoted under each step. The "floating culture with rotation" can be achieved using spinner flasks or 24-well plates placed on an in-incubator orbital shaker.

[00011] FIGS. 2A-2I describe skin organoids generated from mouse pluripotent stem cells (mPSCs). (A) Schematic of inner ear and skin organoid culture. (B) Cryosection of a skin organoid immunostained with anti-cytokeratin-5 (Krt5) and DAPI. (C) $Sox2^{+}$ dermal papilla cells in developing hair bulbs. (D) Cleared organoid with hair shafts extending into the organoid core (arrowheads). (E) Hair follicle protruding from a day 30 skin organoid. (F-H) Parts of hair follicle observed in skin organoids. (I) Quantification of hair follicles per aggregate over time in culture (error bars, min/max). ECT, ectoderm; MES, mesoderm; NNE, non-neural ectoderm; EPI, epidermis; OTP/OTV, otic placode/vesicle; DERM, dermis. Scale bars, 100 (B, D, H), 50 (E, F, G), 25 μm (C).

[00012] FIGS. 3A-3G demonstrate keratinocyte derivation from human pluripotent stem cell (hPSC) aggregates in three-dimensional (3D) culture. (A) Schematic of keratinocyte induction. (B-D) Differential interference contrast (DIC) microscopy images of aggregates on day 0, day 6, and day 20. Note the cellular structures containing cells migrating from the core to the epithelium. (E-F) Cryosection of day 3 aggregates showing non-neural $TFAP2^{+}$ cells in the outer epithelium and underlying tissues. (G) $KRT5^{+}$ $TFAP2^{+}$ keratinocyte-like cells in the epithelium of a day 20 aggregate. Scale bars, 200 (D), 100 (C, E, F), 50 μm (B).

CLAIMS

We claim:

1. A method of obtaining a three-dimensional multilayered human skin composition, the method comprising:

(a) culturing human pluripotent stem cell aggregates in floating culture in a culture medium comprising FGF-2 and SB-43154 for about four days, whereby non-neural epithelium forms within the aggregates;

(b) culturing the cultured aggregates of (a) in a culture medium comprising an FGF and LDN-193189 for eight days;

(c) embedding the cultured aggregates of (b) in a porous substrate comprising extracellular matrix components; and

(d) culturing the embedded aggregates of (c) in floating culture for at least 48 days under conditions that promote self-assembly of cells within the embedded aggregates into the three-dimensional, multilayered human skin composition comprising an epidermal layer, a dermal layer, and a plurality of cells forming a functional hair follicle.

2. The method of claim 1, wherein the porous substrate is a semi-solid culture medium.

3. The method of claim 1, wherein the porous substrate is a three-dimensional (3D) porous biomaterial.

4. The method of claim 3, wherein the three-dimensional (3D) porous biomaterial is a chemically defined hydrogel.

5. The method of claim 1, wherein the extracellular matrix is a basement membrane extract (BME).

6. The method of claim 1, wherein the epidermal layer is in direct contact with the dermal layer.

7. The method of claim 1, wherein the epidermal layer comprises $P63^{+}KRT5^{+}$ epidermal keratinocytes.

8. The method of claim 1, wherein the dermal layer comprises follicle-initiating dermal papilla cells.

9. The method of claim 1, wherein the three-dimensional, multilayered human skin composition comprises a plurality of cells capable of forming a functional hair follicle, wherein the cells capable of forming a functional hair follicle comprise one or more of mesenchymal stem cells, dermal papilla cells, dermal sheath cells, and follicular epidermal stem cells.

10. The method of claim 1, further comprising seeding the porous substrate with one or more cell types selected from the group consisting of epidermal immune cells, mesoderm-derived cells, and endothelial cells.

11. The method of claim 11, wherein the epidermal immune cells are Langerhan's cells.

12 A three-dimensional, multilayered engineered human skin composition comprising an epidermal layer comprising human pluripotent stem cell-derived epidermal keratinocytes, a dermal layer comprising human pluripotent stem cell-derived dermal fibroblasts, a plurality of human pluripotent stem cell-derived cells forming a functional hair follicle,

wherein the epidermal layer and dermal layer are in direct contact.

13. The engineered human skin composition of claim 12, wherein the multilayered engineered human skin composition comprises a plurality of cells capable of forming a functional hair follicle, wherein the cells comprise one or more of mesenchymal stem cells, dermal papilla cells, dermal sheath cells, and follicular epidermal stem cells.

14. The engineered human skin composition of claim 12, further comprising a scaffold.

15. The engineered human skin composition of claim 14, wherein the scaffold is biodegradable or bioabsorbable.

16. The engineered human skin composition of claim 14, wherein the scaffold is a synthetic matrix.

17. The engineered human skin composition of claim 12, wherein the composition is capable of viable transplantation and engraftment to a human subject.

18. A method of testing a compound for effects on hair growth, the method comprising

(a) contacting a test compound to a three-dimensional multilayered human skin composition obtained according to the method of claim 1 or to the three-dimensional, multilayered engineered human skin composition of claim 12; and

(b) detecting an effect of the agent on one or more cell types within the contacted skin composition.

19. The method of claim 18, wherein detecting comprises performing a method selected from the group consisting of RNA sequencing, gene expression profiling, transcriptome analysis, and protein expression analysis.

20. The method of claim 18, wherein the agent is screened for an effect on gene expression and wherein detecting comprises assaying for differential gene expression relative to an uncontacted skin composition.

21. Use of a three-dimensional multilayered human skin composition obtained according to the method of claim 1 or the three-dimensional, multilayered engineered human skin composition of claim 12 in a drug discovery screen.