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 skin composition, the method comprising: (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 forms within the aggregates; (b) embedding the cultured aggregates of (a) in a porous substrate comprising extracellular matrix components; and (c) culturing the embedded aggregates of (b) for at least about 25 days to about 120 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. 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 another embodiment, 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 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, and a plurality of human pluripotent stem cell-derived cells capable of 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 skin composition obtained according to the method of the first aspect; 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 skin composition obtained according to the method of the first aspect 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).