RHE, is required to advance skin culture models for all dermatology applications within pharmaceutical, cosmetic and academic settings.

SUMMARY

The present disclosure provides, in a first aspect, a skin culturing apparatus configured for biphasic culturing of *ex vivo* mammalian skin or a mammalian skin substitute comprising a first chamber, a second chamber, a barrier, and a skin sample holder, wherein: the first chamber is configured to provide a gaseous environment having a relative humidity below 90% and comprising less than 5% CO_2; the second chamber is configured to provide tissue culture medium at a temperature of 33.0-37.5°C and pH of 6.1-7.9; the skin sample holder is configured to house *ex vivo* mammalian skin and/or a mammalian skin substitute; and the barrier separates the first and second chambers, and comprises an orifice ~~is configured~~ to receive the skin sample holder; wherein in use: (i) the first chamber comprises a gaseous environment having a relative humidity below 90% and comprising less than 5% CO_2; (ii) the second chamber comprises a tissue culture medium at a temperature of 33.0-37.5°C and pH of 6.1-7.9; (iii) the skin sample holder houses *ex vivo* mammalian skin and/or a mammalian skin substitute; (iv) a first surface of the *ex vivo* mammalian skin or skin substitute is exposed to the gaseous environment, but not to the tissue culture medium; and (v) a second surface of the *ex vivo* mammalian skin or skin substitute is exposed to the tissue culture medium, but not to the gaseous environment.

The present disclosure provides, in a second aspect, a method of culturing *ex vivo* mammalian skin and/or a mammalian skin substitute, the method comprising providing *ex vivo* mammalian skin and/or a mammalian skin substitute and culturing the *ex vivo* mammalian skin and/or skin substitute using the apparatus of the disclosure. The disclosure also provides *ex vivo* mammalian skin and/or a mammalian skin substitute which has been cultured according to said method.

In another aspect, the present disclosure provides a method for testing the effect of a compound or composition on *ex vivo* mammalian skin and/or a mammalian skin substitute, the method comprising: (a) providing *ex vivo* mammalian skin and/or a mammalian skin substitute; (b) culturing the *ex vivo* mammalian skin and/or skin substitute using a method as described herein; (c) contacting said *ex vivo* mammalian skin and/or skin substitute with the compound or composition; and (d) determining the effect of the compound or composition on the *ex vivo* mammalian skin and/or skin substitute, optionally comprising measuring gene expression levels; optionally wherein the compound or

composition is: (i) a therapeutic compound or composition, e.g. an anti-cancer compound or composition; (ii) a pharmaceutical compound or composition, which optionally comprises a pharmaceutically acceptable carrier; (iii) a cosmetic compound or composition; and/or (iv) a pesticide, further optionally wherein: said effect is the promotion or inhibition of hair growth; said effect comprises the treatment or prevention of a skin-related condition or disease, e.g. dermatitis or melanoma; said effect is an adverse skin reaction, e.g. wherein said method is a method for testing the toxicity of a compound or composition for said adverse skin reaction; and/or said effect is skin sensitization, and/or skin irritation, further optionally wherein the *ex vivo* mammalian skin and/or skin substitute comprises a model of a disease condition or change to healthy skin physiology, e.g. dandruff formation.

~~In yet another aspect, the present disclosure provides *ex vivo* mammalian skin and/or a mammalian skin substitute which has been cultured according to the methods as described herein.~~

The present disclosure provides, in a third example, a method of culturing *ex vivo* mammalian skin and/or a mammalian skin substitute, said method comprising maintaining a first surface of the *ex vivo* mammalian skin or skin substitute in a gaseous environment comprising less than 5% CO_2, at a temperature below 37°C and a relative humidity below 90%; and maintaining a second surface of the *ex vivo* mammalian skin or skin substitute in a tissue culture medium at a temperature of 33.0-37.5°C and pH of 6.1-7.9; wherein the first surface is not exposed to the tissue culture medium; and the second surface is not exposed to the gaseous environment. The disclosure also provides *ex vivo* mammalian skin and/or a mammalian skin substitute which has been cultured according to said method.

The present disclosure provides, in a fourth example, a method for testing the effect of a compound or composition on *ex vivo* mammalian skin and/or a mammalian skin substitute, the method comprising: (a) providing *ex vivo* mammalian skin and/or a mammalian skin substitute; (b) culturing the *ex vivo* mammalian skin and/or skin substitute using a method of the disclosure; (c) contacting said *ex vivo* mammalian skin and/or skin substitute with the compound or composition; and (d) determining the effect of the compound or composition on the *ex vivo* mammalian skin and/or skin substitute.

The present disclosure provides, in a fifth example, a method for assessing the barrier function of *ex vivo* mammalian skin and/or a mammalian skin substitute, the method comprising: (a) providing *ex vivo* mammalian skin and/or a mammalian skin substitute; (b) culturing the *ex vivo* mammalian skin and/or skin substitute using a method

CLAIMS

1. A skin culturing apparatus configured for biphasic culturing of *ex vivo* mammalian skin or a mammalian skin substitute comprising a first chamber, a second chamber, a barrier, and a skin sample holder, wherein:

the first chamber is configured to provide a gaseous environment having a relative humidity below 90% and comprising less than 5% CO_2;

the second chamber is configured to provide tissue culture medium at a temperature of 33.0-37.5°C and pH of 6.1-7.9;

the skin sample holder is configured to house *ex vivo* mammalian skin and/or a mammalian skin substitute; and

the barrier separates the first and second chambers, and ~~is configured~~comprises an orifice to receive the skin sample holder;

wherein in use:

(i) the first chamber comprises a gaseous environment having a relative humidity below 90% and comprising less than 5% CO_2;

(ii) the second chamber comprises a tissue culture medium at a temperature of 33.0-37.5°C and pH of 6.1-7.9;

(iii) the skin sample holder houses *ex vivo* mammalian skin and/or a mammalian skin substitute;

(iv) a first surface of the *ex vivo* mammalian skin and/or skin substitute is exposed to the gaseous environment, but not to the tissue culture medium; and

(v) a second surface of the *ex vivo* mammalian skin and/or skin substitute, which opposes said first surface, is exposed to the tissue culture medium, but not to the gaseous environment.

2. The apparatus of claim 1, wherein:

(a) the first chamber comprises a gaseous environment having a relative humidity below 90% and comprising less than 5% CO_2, wherein optionally said gaseous environment is provided by an inflow of compressed air into the first chamber;

(b) the second chamber comprises tissue culture medium at a temperature of 33.0-37.5°C, preferably at about 37°C, and a pH of 6.1-7.9; and/or

(c) the skin sample holder houses *ex vivo* mammalian skin and/or a mammalian skin substitute.

3. The apparatus of claim 1 or claim 2, wherein the epidermis of *ex vivo* mammalian skin or epidermis-equivalent of a mammalian skin substitute provides the first surface; and/or the dermis of *ex vivo* mammalian skin or dermis-equivalent of a mammalian skin substitute provides the second surface.

4. The apparatus of any of the preceding claims, wherein the *ex vivo* mammalian skin and/or skin substitute comprises stratum corneum, stratum granulosum, stratum spinosum and/or a stratum basale layers.

5. The apparatus of claim 4, wherein in use:

the stratum corneum layer is exposed to the gaseous environment, but not to the tissue culture medium; and/or

the stratum basale layer and/or the dermis or dermal equivalent is exposed to the tissue culture medium, but not to the gaseous environment.

6. The apparatus of any of the preceding claims, wherein the *ex vivo* mammalian skin and/or skin substitute is one of a plurality of *ex vivo* mammalian skin and/or skin substitute samples, wherein the plurality of samples are arranged in separate skin sample holders in an array format.

7. The apparatus of any of the preceding claims, wherein the apparatus comprises:

(a) one or more pumps, which pump tissue culture medium into and/or out of the second chamber, wherein optionally the flow rate is between 1 and 45 ml/min; and/or

(b) one or more pH probe(s), temperature probe(s), CO_2 sensor(s), and/or humidity sensor(s); and/or

(c) a housing that encloses the first and second chambers, optionally wherein the housing additionally comprises a filter outlet, which is configured to enable the release of gases; and/or

(d) an inflow pipe which is configured to deliver gases into the first chamber, wherein optionally said gases comprise compressed air; and/or

(e) a sample of *ex vivo* mammalian skin or a mammalian skin substitute, optionally wherein the skin substitute is recombinant human epidermis (RHE) or full thickness RHE (FT RHE).

8. The apparatus of any of the preceding claims, wherein the second chamber comprises tissue culture medium and said tissue culture medium is maintained at a temperature of 33.0-37.5°C by the action of a heater.

9. The apparatus of any of the preceding claims, wherein said apparatus provides monitoring and feedback control of culture media pH, humidity and/or temperature.

10. The apparatus of any of the preceding claims, wherein:

(a) immune cells are retained in the *ex vivo* mammalian skin and/or skin substitute following at least five days in culture; and/or

(b) epidermal cells of the *ex vivo* mammalian skin and/or skin substitute retain a normal lipid profile following at least three days in culture; and/or

(c) cells of the *ex vivo* mammalian skin and/or skin substitute express keratinocyte differentiation markers at normal detectible levels following at least five days in culture; and/or

(d) cells of the *ex vivo* mammalian skin and/or skin substitute do not have elevated K16 expression following at least five days in culture relative to baseline levels.

11. A method of culturing *ex vivo* mammalian skin and/or a mammalian skin substitute, the method comprising providing one or more samples of *ex vivo* mammalian skin and/or a mammalian skin substitute and culturing the one or more samples using the apparatus according to any one of the preceding claims.

12. The method of claim 11, wherein said method comprises:

maintaining a first surface of the *ex vivo* mammalian skin or skin substitute in a gaseous environment comprising less than 5% CO_2, at a temperature below 37°C and a relative humidity below 90%; and

maintaining a second surface of the *ex vivo* mammalian skin or skin substitute in a tissue culture medium at a temperature of 33.0-37.5°C and pH of 6.1-7.9;

wherein the first surface is not exposed to the tissue culture medium; and the second surface is not exposed to the gaseous environment.

13. The method of claim 11 or claim 12, wherein the skin substitute is FT RHE or RHE.

14. The method of any of claims 11-13, wherein the gaseous environment:

(a) comprises 0.02-0.05% CO_2; and/or

(b) has a relative humidity of 40-50% RH; and/or

(c) is at a temperature of 18-25°C; and/or

(d) is sterile.

15. The method of any of claims 11-14, wherein the tissue culture medium:

(a) flows under the dermis of the *ex vivo* skin or dermal equivalent of the FT-RHE at a flow rate ranging from 1 ml/min to 45 ml/min; and/or

(b) is at a temperature of 37°C; and/or

(c) is maintained at pH 6.1-7.9

16. The method of any of claims 11-15, wherein:

(a) immune cells are retained in the *ex vivo* mammalian skin and/or skin substitute following at least five days in culture;

(b) epidermal cells of the *ex vivo* mammalian skin and/or skin substitute retain a normal lipid profile following at least three days in culture;

(c) cells of the *ex vivo* mammalian skin and/or skin substitute express keratinocyte differentiation markers at normal detectible levels following at least five days in culture; and/or

(d) cells of the *ex vivo* mammalian skin and/or skin substitute do not have elevated K16 expression following at least five days in culture relative to baseline levels.

17. The method of any of claims 11-16, wherein said method comprises monitoring and feedback control of the pH, humidity, temperature, and/or flow rate of tissue culture medium.

18. A method for testing the effect of a compound or composition on *ex vivo* mammalian skin and/or a mammalian skin substitute, the method comprising:

(a) providing *ex vivo* mammalian skin and/or a mammalian skin substitute;

(b) culturing the *ex vivo* mammalian skin and/or skin substitute using a method according to any one of claims 11-16;

(c) contacting said *ex vivo* mammalian skin and/or skin substitute with the compound or composition; and

(d) determining the effect of the compound or composition on the *ex vivo* mammalian skin and/or skin substitute, optionally comprising measuring gene expression levels;

optionally wherein the compound or composition is:

(i) a therapeutic compound or composition, e.g. an anti-cancer compound or composition;

(ii) a pharmaceutical compound or composition, which optionally comprises a pharmaceutically acceptable carrier;

(iii) a cosmetic compound or composition; and/or

(iv) a pesticide,

further optionally wherein:

said effect is the promotion or inhibition of hair growth;

said effect comprises the treatment or prevention of a skin-related condition or disease, e.g. dermatitis or melanoma;

said effect is an adverse skin reaction, e.g. wherein said method is a method for testing the toxicity of a compound or composition for said adverse skin reaction; and/or

said effect is skin sensitization, and/or skin irritation,

further optionally wherein the *ex vivo* mammalian skin and/or skin substitute comprises a model of a disease condition or change to healthy skin physiology, e.g. dandruff formation.

~~19. *Ex vivo* mammalian skin and/or a mammalian skin substitute which has been cultured according to the methods of any of claims 11-18.~~

~~20~~19. The apparatus according to any of claims 1-10 or a method according to any of claims 11-18, wherein:

(a) the barrier comprises a plastic and/or silicon gasket;

(b) the skin sample holder comprises a mesh and optionally the *ex vivo* skin or skin substitute is locatable on said mesh;

(c) the skin sample holder is a transwell insert;

(d) the skin sample or skin equivalent is: embedded in or surrounded by a gel; supported by a gel; or is inside a transwell;

(e) cells retain metabolic activity/viability for at least 5 days;

(f) epidermal skin surface properties of pH and moisture are maintained for 7 days;

(g) the tissue culture medium flows through the second chamber at a flow rate ranging from 0.01 ml/min to 45 ml/min;

(h) the tissue culture medium is at a temperature of 33-37°C;

(i) the tissue culture medium is maintained at pH 6.1-7.9; and/or

(j) the tissue culture medium is maintained at an oxygen saturation level of 0.2-15% O_2, e.g. by using monitoring and/or feedback control;

(k) said method comprises monitoring and/or feedback control of CO_2, humidity, temperature and/or flow rate within the gaseous environment;

(l) the first surface is an epidermal cornified layer and/or the second surface is the dermis and/or hypodermis;

(m) the epidermal cornified layer thickness of said *ex vivo* mammalian skin and/or skin substitute remains constant or substantially constant (e.g. within 1, 2, 3, 4, 5%, 7%, 10%, 15%, 20% or 25%), relative to said thickness at day 0, during at least 1, 2, 3, 4, 5, 6, or 7 days of culture in said apparatus;

(n) the epidermal cornified layer thickness of said *ex vivo* mammalian skin and/or skin substitute remains constant, relative to said thickness at day 0, during at least 7 days of culture in said apparatus; and/or

(o) said apparatus comprises one or more sensors to assess skin function and biological outputs, wherein optionally said biological output comprises impedance for skin barrier and/or tissue viability, transepidermal water loss, skin surface pH, intra- and intercellular pH, cytokines, gases (tissue oxygen, nitric oxide), glucose, small molecules, and/or peptide/protein sensors.