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 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

of the disclosure; (c) contacting said *ex vivo* mammalian skin and/or skin substitute with the compound or composition; and (d) determining to what extent the compound or composition can pass through the *ex vivo* mammalian skin and/or skin substitute.

The present disclosure provides, in a sixth example, a method for assessing immune function and/or inflammation in *ex vivo* mammalian skin and/or a mammalian skin substitute, 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 an allergen or immunogen; and (d) determining whether an immune response or inflammation of the *ex vivo* mammalian skin and/or skin substitute occurs.

The present disclosure provides, in a seventh example, a method for assessing the genomic, transcriptomic, metabolomic, lipidomic and/or proteomic response of *ex vivo* mammalian skin and/or a mammalian skin substitute to a stimulus, 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) exposing said *ex vivo* mammalian skin and/or skin substitute to a stimulus; and (d) determining the genomic, transcriptomic, metabolomic, lipidomic and/or proteomic response of the *ex vivo* mammalian skin and/or skin substitute to said stimulus.

The present disclosure provides, in an eighth example, a method for conducting genomic, transcriptomic, metabolomic, lipidomic and/or proteomic analysis of *ex vivo* mammalian skin and/or a mammalian skin substitute, 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 the disclosure; (c) conducting genomic, transcriptomic, metabolomic, lipidomic and/or proteomic assays on the *ex vivo* mammalian skin and/or skin substitute.

The present disclosure provides, in an ninth example, 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 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, wherein the apparatus comprises: (a) a housing that encloses the first and second chambers; (b) an inflow pipe which is configured to deliver compressed air or medical air into the first chamber; (c) a filter outlet which is configured to enable the release of gases; (d) one or more pumps which pump tissue culture medium into and/or out of the second chamber, e.g. at a flow rate of between 1 and 45 ml/min; (e) one or more CO_2 sensor(s), temperature probe(s) and/or humidity sensor(s) in the first chamber; (f) one or more temperature probe(s) in the second chamber; (g) means for maintaining the tissue culture medium at a controlled depth within the second chamber; and/or (h) means for providing feedback control of humidity, temperature and/or tissue culture medium pH.

The present disclosure provides, in an tenth example, a method for assessing dermal absorption 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 according to the disclosure; (c) contacting said *ex vivo* mammalian skin and/or skin substitute with a compound or composition; and (d) determining to what extent the compound or composition is absorbed by the *ex vivo* mammalian skin and/or skin substitute.

The present disclosure provides, in a eleventh example, a method for assessing skin sensitization and/or skin irritation in an *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 the disclosure; (c) contacting said *ex vivo* mammalian skin and/or skin substitute with a compound or composition or exposing said *ex vivo* mammalian skin and/or skin substitute to irradiation; and (d) determining whether skin sensitization and/or skin irritation occurs in the *ex vivo* mammalian skin and/or skin substitute.

The present disclosure provides, in a twelfth example, a method for testing the effect of a gas, aerosol or pollutant 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 the disclosure; (c) contacting said *ex vivo* mammalian skin and/or skin substitute with said gas, aerosol or pollutant; and (d) determining the effect of said gas, aerosol or pollutant on the *ex vivo* mammalian skin and/or skin substitute.

The present disclosure provides, in a thirteenth example, *ex vivo* mammalian skin and/or a mammalian skin substitute which has been cultured according to methods of the disclosure.

The present disclosure provides, in a fourteenth example, a method for testing the effect of air pollution and/or radiation 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 the disclosure; (c) delivering an environmental/ atmospheric source of pollution to the ex vivo mammalian skin and/or skin substitute and/or exposing the mammalian skin and/or mammalian skin substitute to radiation; and (d) analyzing the effect of step (c) on skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic or lipidomic responses, wherein optionally said radiation is electromagnetic and/or ionizing radiation.

The present disclosure provides, in a fifteenth example, a method for assessing the effect of low oxygen levels 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 the disclosure, wherein the oxygen levels is the gaseous environment and/or tissue culture medium are maintained at less than 21%, optionally at less than 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1%; (d) analyzing the effect of said low oxygen levels on skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic or lipidomic responses.

The present disclosure provides, in a sixteenth example, a method for assessing the effect of a medical aesthetic device and/or wearable medical device 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 the disclosure; wherein a medical aesthetic device and/or wearable medical device is used on said ex vivo mammalian skin and/or skin substitute; (e) analyzing the effect of said medical aesthetic device or wearable medical device on skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic or lipidomic responses.

The present disclosure provides, in a seventeenth example, a method for assessing the efficacy and/or safety of skin implants, 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 the disclosure; (c) delivering said skin implant to mammalian skin and/or a mammalian skin substitute; (d) analyzing the effect of said skin implant on skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic or lipidomic responses, wherein optionally said implant is a microchip, e.g. an NFC and RFID chips, a chemical or tattoo sensor, a topical and/or an injectable sensor.

The present disclosure provides, in a eighteenth example, a method for repeat dose testing of a chemical, compound or aerosol, 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 the disclosure; (c) dosing of a chemical, compound or aerosol, which is repeated more than once and/or over a period longer than 24h, to the *ex vivo* mammalian skin and/or a mammalian skin substitute; and (d) analyzing the skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic and lipidomic responses before, during and/or after the skin exposure to repeat dosing of a chemical, compound or aerosol.

The present disclosure provides, in a nineteenth example, a method for assessing responses to disruption of ex vivo mammalian skin and/or skin substitute, such as wound healing, wherein the method comprises: (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 the disclosure; (c) disrupting the epidermis and/or dermis of the ex vivo mammalian skin and/or skin substitute, for example by punch biopsy, cutting or abrasion; and (d) analyzing the skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic and lipidomic responses before, during and/or after the skin wound healing response.

The present disclosure provides, in a twentieth example, a method for assessing the skin microbiome and/or effects of bacterial, fungal, viral exposure to skin, 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 the disclosure; (c) assessing skin microbiome levels in said *ex vivo* mammalian skin and/or skin substitute; (d) introducing bacteria, fungi and/or viruses to the apparatus; (e) analyzing the effect of said bacteria, fungi and/or viruses on skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic or lipidomic responses.

The present disclosure provides, in a twenty-first example, a method for assessing skin permeation, corrosion, pigmentation and/or photosensitivity 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 according to the disclosure; (c) exposing the *ex vivo* mammalian skin and/or skin substitute to a chemical, a compound and/or a form of assault or irradiation; and (d) assessing skin permeation, corrosion, pigmentation and/or photosensitivity of the *ex vivo* mammalian skin and/or a mammalian skin substitute.

The present disclosure provides, in a twenty-second example, a method for developing computational analysis of skin function and metabolism, 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 the disclosure; (c) assessing skin function and metabolism; and (d) conducting computational analysis of data outputs for interpreting and predicting skin function and metabolism.

Examples of the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, sixteenth, seventeenth, eighteenth, nineteenth, twentieth, twenty-first or twenty-second examples include the following features:

(i) (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 or medical air into the first chamber; and/or (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;

(ii) said 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;

(iii) the apparatus comprises *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);

(iv) the *ex vivo* mammalian skin and/or skin substitute comprises stratum corneum, stratum granulosum, stratum spinosum and/or a stratum basale layers;

(v) 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;

(vi) 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;

(vii) the apparatus comprises 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;

(viii) the apparatus comprises one or more pH probe(s), temperature probe(s), CO_2 sensor(s), and/or humidity sensor(s);

(ix) in use the tissue culture medium is maintained at a controlled depth within the second chamber;

(x) the barrier comprises a gasket, e.g. a silicon gasket, or an o-ring;

(xi) 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;

(xii) said apparatus provides monitoring and feedback control of culture media pH, humidity and/or temperature;

(xiii) the apparatus comprises a housing that encloses the first and second chambers;

(xiv) the apparatus comprises an inflow pipe which is configured to deliver gases into the first chamber, wherein optionally said gases comprise compressed air or medical air; and/or the housing additionally comprises a filter outlet, which is configured to enable the release of gases.

(xv) (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;

(xvi) the skin substitute is FT RHE or RHE;

(xvii) 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.

(xviii) 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;

(xix) (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;

(xx) said method comprises monitoring and feedback control of the pH, humidity, temperature, and/or flow rate of tissue culture medium;

(xxi) 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; (iv) a chemical substance; and/or (v) a pesticide.

(xxii) 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 (e.g. a cosmetic compound or composition) for said adverse skin reaction; and/or said effect is skin sensitization, and/or skin irritation;

(xxiii) step (d) comprises measuring gene expression levels;

(xxiv) the *ex vivo* mammalian skin and/or skin substitute comprises a model of a disease condition;

(xxv) said skin substitute comprises immune cells, wherein optionally said skin substitute is RHE or FT RHE;

(xxvi) said apparatus comprises microfluidics and/or means for facilitating fluidic movement of the tissue culture medium that is not regulated by a pump;

(xxvii) in use, the hypodermis or adipose tissue is exposed to the tissue culture medium, but not to the gaseous environment;

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

(xxix) the cells retain metabolic activity/viability for at least 5 days without the need of a culture media that is metabolically active (e.g. culture media that reacts with MTT to create formazan salt that is purple in colour);

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

(xxxi) the gaseous environment comprises 0.02-0.09% CO_2;

(xxxii) the method comprises analyzing the effect of the compound or composition on skin immune, inflammatory, toxicology, viability, metabolism, barrier function, genetic, epigenetic, proteomic or lipidomic responses;

(xxxiii) the composition comprises an injectable composition, e.g. a vaccine composition, a dermal filler, PRP (platelet rich protein), botox, etc.;

(xxxiv) the barrier comprises a plastic gasket;

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

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

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

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

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

(xl) the tissue culture medium flows through the second chamber at a flow rate ranging from 0.01 ml/min to 45 ml/min, e.g. 0.01-10 ml/min, 0.02-10 ml/min, 0.03-10 ml/min, 0.05-10 ml/min, 0.06-10 ml/min, 0.1-10 ml/min, 0.5-10 ml/min, 0.5-1 ml/min;

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

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

(xliii) 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;

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

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

(xlvi) said *ex vivo* mammalian skin and/or skin substitute 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;

(xlvii) said housing is configured to allow or block the passage of all or some wavelengths of electromagnetic and/or ionising radiation, including UV radiation;

(xlviii) the gaseous environment has a relative humidity below 80%, e.g. below 75%, 70%, 65%, 60%, 55%, 50%, 45%, etc.;

(xlix) the gaseous environment comprises 0.03-0.09% CO_2;

(l) the gaseous environment has a temperature below 29°C;

(li) the gaseous environment comprises medical air, atmospheric air, environmental air, polluted air, an aerosol, etc.;

(lii) the first chamber comprises desiccant(s) to aid the regulation of humidity;

(liii) flow of gases into the first chamber is regulated by an on/off valve (e.g. for pressurized gas) or a pump, fan, and/or vacuum (e.g. for non-pressurized gas), e.g. to aid gas exchange;

(liii) the apparatus comprises a heater, which may be a hotplate, heating element, heatblock and/or a water bath;

(liii) the *ex vivo* mammalian skin or skin substitute may be healthy or diseased skin;

(liv) 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;

(lv) 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; and

(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.

For the avoidance of doubt, the examples described herein can be combined unless context clearly dictates otherwise. Furthermore, the first, second, third, fourth, fifth, sixth, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, sixteenth, seventeenth, eighteenth, nineteenth, twentieth, twenty-first or twenty-second examples, and

the examples thereof can be combined with other optional features of the present disclosure as disclosed elsewhere herein unless context clearly dictates otherwise.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: A skin culturing apparatus configured for biphasic culturing of *ex vivo* mammalian skin or FT RHE. Figure 1A & B. 1A. Detailed schematic of the plastic ware and attachments of the laminar flow hood fluidic skin culture device; 1B Component parts of laminar flow hood fluidic skin culture device. Figure 1B & 1D. Schematic of a device for *ex vivo* and FT-RHE skin culture with continuous monitoring and feedback control of culture media pH, regulated between pH 7.2 and 7.4 by a valve that opens to allow the inflow of CO_2 if the pH increases too much. Fluidic culture media flow- ranging from 3.3ml/min to 41.67 ml/min in a closed loop system. Culture media temperature is regulated at 37°C (33-37°C) on a hotplate. A silicon gasket and/or an o-ring and plastic holders separate the fluidic tissue culture media phase, which the dermal side of the skin tissue is exposed to, from the atmospheric air phase to which the apical side of the epidermis is exposed. A silicon gasket and/or an o-ring may also isolate the temperature and pH sensors and the sample supports from the gas phase. The entire system is placed in a laminar flow hood to maintain sterility and atmospheric air is dependent upon ambient air conditions within the room/laminar flow hood. Figure 1C & 1E. An autoregulated benchtop device for *ex vivo* and FT-RHE skin culture with continuous monitoring and feedback control of 1. culture media pH, regulated between pH 7.2 and 7.4; 2. atmospheric air control linked to humidity monitors to maintain humidity between 40-45% RH. The pH is regulated by linking a pH probe that continuously monitors culture media pH to a CO_2 valve that opens

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 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. 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.