(51) International Patent Classification:
C12M 3/00 (2006.01)  C12N 3/06 (2006.01)

(21) International Application Number:
PCT/IB2005/053770

(22) International Filing Date:
15 November 2005 (15.11.2005)

(25) Filing Language:
English

(26) Publication Language:
English

(30) Priority Data:
04 52626 15 November 2004 (15.11.2004) FR
60/631,191 29 November 2004 (29.11.2004) US

(71) Applicant (for all designated States except US):
LOREAL [FR/FR]; 14 rue Royale, F-75008 PARIS (FR).

(72) Inventor; and

(75) Inventor/Applicant (for US only): LEVEQUE,
Jean-Luc [FR/FR]; 29 avenue de Wagram, F-75017 PARIS (FR).


(54) Title: A DEVICE COMPRISING A SAMPLE OF RECONSTRUCTED TISSUE AND A DETECTION SYSTEM

(57) Abstract: The present invention relates to a device (1) comprising: a sample of reconstructed tissue (4); and a detection system (20), (30) that is at least partially in contact with the tissue sample and/or with a device serving to culture said sample, said detection system making it possible to measure the influence of a physical, biological, and/or chemical stress on at least a fraction of the sample, or vice-versa.
A DEVICE COMPRISING A SAMPLE OF RECONSTRUCTED TISSUE AND A DETECTION SYSTEM

The present invention relates to devices associating a sample of reconstructed tissue, e.g. of reconstructed skin, with a detection system.

Several types of reconstructed skin are known that are characterized by the similarity of their epidermis with natural skin.

A first epidermis model contains keratinocytes exclusively. The use of that model makes it possible, amongst other things, to simulate aging of the skin.

A second epidermis model also contains melanocytes. That model is pigmented and makes it possible to understand better the phenomenon of melanogenesis.

A third epidermis model contains keratinocytes, melanocytes, and Langerhans cells. That epidermis model makes it possible to understand the phenomenon of photoimmunosuppression, or the immune response of the skin in the event of an allergy, for example.

Reconstructed skin can be obtained by using a culture device to culture human-adult keratinocytes on a collagen base, under conditions that enable them to differentiate and to reconstruct an epidermis provided with a functional corneum. The keratinocytes can be subjected to a stage of culture while immersed on a collagen support, and can then be kept immersed over a time period that makes it possible to obtain differentiation of the epidermis and the formation of a corneum.

Reconstructed skin is sold under the trade name Episkin®, for example.

In order, in particular, to enable new substances for treating the skin and/or for coming into contact therewith to be developed, it is necessary to make it easier to understand the biological, chemical, or physical characteristics of the skin.

There also exists a need to improve and/or to facilitate testing the safety and/or effectiveness of such substances.
In a first of its aspects, the invention provides a device comprising:

- a sample of reconstructed tissue, and in particular of reconstructed skin; and
- a detection system that is at least partially in contact with the sample and/or with a device serving to culture said sample, said detection system making it possible to measure the influence of a physical, biological, and/or chemical stress on at least a fraction of the sample, or vice-versa.

In one exemplary embodiment, said stress is an electrical, thermal, optical, chemical and/or biological stress.

In another exemplary embodiment, said detection system includes a sensor in contact with said sample.

In an embodiment of the invention, the detection system is configured to measure the influence of a physical, biological, and/or chemical stress on at least a fraction of the sample.

In another embodiment of the invention, the detection system is configured to measure the influence of at least a fraction of the sample on a physical, biological, and/or chemical stress.

The detection system may include a sensor and/or stress-impacting means in contact with the sample and/or in contact with the device serving to culture it.

Throughout the description and in the claims, the term "sensor" should not be understood in a narrow sense, but encompasses a variety of components that, on their own or in association with other means, are capable of supplying a useful signal in response to a physical, chemical, and/or biological stress being imparted to the sample. In particular, the detection system may include a sensor that is secured to the sample, while said sample is viable.

The expression "stress-impacting means" should likewise be understood broadly, and encompasses components that are, for example, capable of modifying at least one physical,
chemical, and/or biological parameter of the sample, e.g. of generating a physical stress, in particular electrical, thermal, optical, mechanical, chemical, and/or biological, e.g. by releasing a chemical and/or biological entity.

The detection system may include various sensors, e.g.:
- a sensor for detecting amino acids; vitamins; enzymes, in particular glucose dehydrogenase enzymes, glucamate dehydrogenase enzymes, and/or NADH oxidase enzymes; acetylcholine; and/or oxygenated water;
- a sensor for detecting partial pressure of at least one gas, in particular a $P_{CO2}$, $P_{CO2}$ sensor;
- a sensor that is sensitive to the concentration of at least one ionic species, in particular a pH sensor;
- a biological-tissue sensor;
- at least one electrode in contact with the sample, said electrode possibly including, where appropriate, a material on its surface that is inert relative to the sample, in particular a passivated material;
- one or more sensors and/or optical stress-imparting means, e.g. at least one of: an optrode; a photodiode; a phototransistor; a photoconductor; a laser; an optical fiber; and/or a bundle of optical fibers in contact with the reconstructed-skin sample; in particular a bundle of optical fibers or other means arranged to image a fraction of the sample and/or to analyze and/or to locate a chemical or biological entity in said sample;
- a temperature sensor; and
- a heating or cooling source enabling the sample to be heated and/or cooled, e.g. a Peltier-effect component, this list not being limiting.

In an embodiment of the invention, the detection system includes at least a source emitting UVa radiation and/or UVb radiation, and an optical sensor that is sensitive to ultraviolet radiation, in particular to UVa radiation and/or to UVb radiation.

Such a detection system can be useful, in particular so as to ascertain how much ultraviolet radiation is absorbed by
the various layers of the sample, and so as to test new sun
screens, for example.

In another embodiment of the invention, the detection
system includes a light source enabling the sample to be
exposed to light having a wavelength that is selected so as
to excite a fluorescent marker, and a sensor that is
sensitive to the wavelength of the light emitted by the
fluorescent marker.

The reconstructed skin may comprise an epidermis
containing keratinocytes. The reconstructed skin may
optionally include a corneum. The reconstructed skin may
contain melanocytes and/or Langerhans cells. The
reconstructed skin preferably comprises both a dermis and an
epidermis.

As indicated above, at least part of the detection
system may be in contact with the sample.

By way of example, for a reconstructed-skin sample, at
least part of the detection system may be positioned inside
the epidermis, and in particular said system may include a
sensor positioned, at least in part, inside the epidermis,
e.g. below the corneum, in said corneum, or at the interface
between the corneum and the keratinocytes.

Alternatively, at least part of the detection system may
be positioned between the dermis and the epidermis, and in
particular said system may include a sensor positioned
between the dermis and the epidermis.

At least part of the detection system may be positioned
inside the dermis, and in particular the system may include a
sensor positioned inside the dermis.

At least part of the detection system may be positioned
above or below the sample, and in particular the system may
include a sensor positioned above or below the sample. When
the detection system is positioned, at least in part, above
the skin sample, the detection system may serve as a support
for culturing the tissue cells.

Also, the detection system need not be in contact with
the sample, being solely in contact with the culture device.
The detection system may thus include a sensor that is separated from the sample solely by a layer of air, in particular when the sensor is an optical sensor disposed above the sample. The detection system may also include a sensor that is separated from the sample by at least one wall that does not belong to the detection system, in particular a wall belonging to the culture device.

The sample may present a greater or lesser area, e.g. lying in the range about 0.3 square centimeters (cm²) to about 1.35 cm², and in particular in the range about 0.38 cm² to about 1.12 cm².

The culture device may include a basket in which the sample is disposed, and a well to which the basket can be fastened in removable manner. The term "basket" should not be understood in a restrictive sense, and encompasses any sample support that is disposed, at least in part, in the well.

Where appropriate, the detection system may include a sensor that is disposed in at least one of the following locations: below the well; at the bottom of the well; in the thickness of a wall of the well, in particular the bottom wall of the well; below the basket; at the bottom of the basket; and/or in the thickness of a wall of the basket, in particular the bottom wall of the basket. The detection system may also include a sensor that is secured to a support fastened to one of: the well; and the basket. The support may be configured to be removable, where appropriate.

The detection system may include means for processing signals coming from a sensor and/or an interface for connection to a micro-computer.

In another of its aspects, the invention also provides, a method of measuring at least one physical, chemical, and/or biological parameter of at least one reconstructed-tissue sample, e.g. a reconstructed-skin sample, using a device as defined above, said method comprising the following steps:

· exposing the sample to at least one physical, chemical, and/or biological stress; and
using the detection system to measure at least one physical, chemical, and/or biological parameter of the sample during and/or after said stress.

Before the sample is exposed to the stress, the value of the parameter may be measured. By way of example, said parameter may be the concentration of at least one chemical and/or biological entity which may be selected from: amino acids; vitamins; acetylcholine; oxygenated water; O₂, CO₂, H⁺; and/or enzymes, in particular glucose dehydrogenase enzymes, glutamate dehydrogenase enzymes, and/or NADH oxidase enzymes, this list not being limiting.

The stress may include exposing the sample to light radiation, in particular radiation comprising UVA radiation and/or UVB radiation. In this event, it is possible, for example, to measure the concentration of a chemical or a biological species whose presence is associated with light radiation, e.g. free radicals.

The skin may also be exposed to stress that is not optical, but that is thermal and/or mechanical.

The stress may include, or may be preceded by, applying a substance to the sample, in particular a cosmetic or a care product, e.g. a sun screen.

At least two measurements of said parameter may be taken, separated by a time interval, and they may possibly be compared, so as to determine the change in said parameter during stress.

In another of its aspects, the invention also provides a method of measuring the influence of at least one sample of reconstructed tissue of a device as defined above, on a physical, chemical, and/or biological stress parameter, said method comprising the following steps:

- disposing the reconstructed-tissue sample, e.g. a reconstructed-skin sample, in such a manner as to enable it to influence the physical, chemical, and/or biological stress; and

- using the detection system to collect at least one item of information that is representative of the influence
of the sample on said stress.

The stress may be physical stress. By way of example, the stress may include exposing the sample to light radiation, in particular radiation comprising UVa radiation and/or UVb radiation. It is thus possible to measure a magnitude that is representative of the absorption, by the sample, of at least a fraction of the spectrum of the light radiation.

The physical stress may include at least one mechanical action, e.g. sending at least one ultrasound wave onto the sample and/or exposing said sample to at least one electrical stimulation, e.g. so as to measure a mechanical parameter, in particular elasticity, or an electrical parameter, e.g. electrical conductivity.

Where appropriate, at least part of the detection system may serve as a support for culturing the reconstructed tissue. The detection system may thus be present from the start of culturing the tissue, in particular skin. The detection system may possibly receive a surface treatment in order to passivate it so that it does not hinder the growth of the cells and does not damage said cells.

In a variant, the detection system may be implanted while the tissue is being cultured, or it may be implanted in tissue that is completely reconstructed and viable. By way of example, at least part of the detection system may be implanted in the reconstructed tissue only while said system is being used, for example. In particular, the implantation of the detection system may take place between two measurements of a given parameter, for example.

The dimensions of the detection system may be adapted to enable it to be implanted in the sample, without substantially destroying said sample.

In another of its aspects, the invention also provides, a method of measuring at least one physical, chemical, and/or biological parameter of a sample of reconstructed tissue, in particular of reconstructed skin, in which the sample is subjected to a physical, chemical, and/or biological stress,
and in which at least one measurement is taken of the parameter in the presence of the stress.

In one exemplary embodiment, said stress is an electrical, thermal, optical, chemical and/or biological stress.

By way of example, it is possible firstly to expose the sample to light radiation, and secondly to measure a magnitude that is representative of the absorption of said light radiation, by the sample.

The invention can be better understood on reading the following detailed description of non-limiting embodiments thereof, and on examining the accompanying drawings, in which:

· Figure 1 is a diagrammatic axial section of a known culture device for culturing a sample of reconstructed skin;
· Figure 2 is a block diagram of an example of a detection system; and
· Figures 3 to 15 are diagrammatic and fragmentary axial sections showing various ways in which the detection system can be disposed relative to the reconstructed-skin sample and/or to the culture device.

Figure 1 shows a known device for culturing a reconstructed-skin sample 4, also sometimes referred to as a skin equivalent, said device comprising a well 2 receiving a basket 3 carrying the sample 4.

The well 2 can form part of a plate having a plurality of wells, e.g. at least ten.

In the embodiment shown, the well 2 is partially filled with a solution 5, e.g. a nutrient medium.

The sample 4 can comprise an epidermis 6 and a dermis 7, said dermis comprising collagen, for example.

In the embodiment described, the basket 3 includes a bottom wall 13 supporting the skin sample 4. The bottom wall 13 has through perforations 9.

By way of example, the reconstructed-skin sample is cultured in accordance with one of the methods described in

In accordance with the invention, a detection system 10 is associated with the sample 4.

Figure 2 shows the possibility of the detection system 10 including a sensor 20 and/or a stress-impacting means 30, and means 40 for processing signals delivered by the sensor(s) 20, and for controlling the one or more stress-impacting means 30.

By way of example, the above-mentioned means 40 comprise specialized electronic circuits that are adapted to the nature(s) of the sensor(s) and/or the stress-impacting means 30.

The detection system 10 can include an integrated interface 50, making it possible to exchange data with a processor unit 60, which is constituted by a micro-computer, for example. By way of example, the interface 50 is an analog-to-digital interface, a series or parallel interface, a USB interface, or any other interface that makes it possible to exchange data with the micro-computer 60.

The detection system 10 includes one or more sensors 20 and/or stress-impacting means 30 disposed in contact with the skin sample 4 or with the culture device 2, 3.

For the purpose of clarity of the drawing, the sensor 20 and stress-impacting means 30 are represented diagrammatically in the figures by rectangles. It goes without saying that their shapes and dimensions can be various, and in particular can be adapted to be incorporated in the sample without substantially damaging said sample.

In addition, single rectangles 20 and 30 are shown, but the sensor 20 and the stress-impacting means 30 can each comprise a plurality of distinct components that are distributed in the skin sample and/or in or on the culture device 2, 3.

In the embodiment in Figure 3, the sensor 20 and/or the stress-impacting means 30 is/are disposed, at least in part, between the dermis 7 and the epidermis 6; in the embodiment
in Figure 4, the sensor 20 and/or the stress-imparting means 30 is/are disposed, at least in part, inside the epidermis 6, e.g. in the corneum, in the keratinocytes, or at the interface between the corneum and the keratinocytes; and in the embodiment in Figure 5, the sensor 20 and/or the stress-imparting means 30 is/are disposed, at least in part, inside the dermis 7.

In the embodiments in Figures 6 and 7, the sensor 20 and/or the stress-imparting means 30 is/are disposed below the skin sample 4. In this event, at least part of the detection system 10 is fastened to the bottom wall 13 before the stage of culturing the skin.

In the embodiment in Figure 7, the detection system 10 serves entirely to support the skin sample. In particular, it is possible, in this way, to make a device in which a sensor that is sensitive to UV radiation serves, possibly after passivation, as a support to a keratinocyte culture having a stratum corneum on its surface. When such a device is placed below a UV lamp or a sun simulator, it can be particularly useful for measuring a flux of UVa radiation and/or of UVb radiation inside the epidermis, thereby making it possible to characterize the protective effects of a treatment, for example.

When the detection system 10 includes a sensor 20 that is disposed as shown in Figures 3 to 7, the sensor 20 can, for example, make it possible to detect and/or to quantify at least one chemical and/or biological entity that is present in contact therewith or in the vicinity thereof, e.g. an optionally-dissolved gas, reducing agent, oxidizing agent, ion, enzyme, and/or amino acid.

By way of example, the sensor 20 can be an enzyme sensor or a biological-tissue sensor.

By way of example, the sensor 20 can serve to detect amino acids, vitamins, acetylcholine, oxygenated water, the partial pressure of oxygen, the partial pressure of CO₂, and/or enzymes, in particular glucose dehydrogenase, glucamate dehydrogenase, and/or NADH oxidase, this list not
being limiting

The sensor 20 can also serve to measure the pH of a given ionic species.

The sensor 20 can include one or more electrodes, and in particular at least one electrode made out of a material that is inert relative to the reconstructed-skin sample, e.g. a rare metal such as platinum, with the electrode serving to measure an electric current or a voltage, for example.

The sensor 20 can also be an optical sensor that is sensitive to ultraviolet radiation, for example, as mentioned above. By way of example, the sensor 20 includes a component such as a photodiode, a phototransistor, or a photoconductor. It can also be connected to one or more optical fibers, each having one end in contact with the sample.

By way of example, the sensor 20 can include a bundle of optical fibers each having a first end that is in contact with the sample, so as to obtain an image at the other end, e.g. an image of the corneocytes or of other cells in the sample, or an image that makes it possible to locate and/or to quantify a fluorescent compound in the sample.

The sensor 20 can also include means that make it possible to analyze and/or to locate a chemical and/or biological entity, e.g. means that make it possible to quantify an analyte by stimulated electroluminescence, in particular by using a bundle of optical fibers as described in the publication "A. Chovin et al, Development of an ordered array ..., Annal. Chem. 2004, 76, 357-364"

The sensor(s) 20 can be secured to the skin sample when the skin is viable, or it can be fitted to said skin sample prior to use, so as to measure at least one physical parameter.

By way of example, when the skin sample is secured to one or more sensors 20, it is possible to culture the skin sample in the presence of said sensor(s). By way of example, it is possible to deposit keratinocytes on the surface of the sensor, said sensor possibly initially being passivated and then supporting culturing, with the sensor and the culture
being immersed in a culture medium.

Also, the detection system 10 need not be directly in contact with the skin sample 4.

In the embodiment in Figure 9, the detection system 10 includes a sensor 20 and/or stress-imparting means 30 disposed, at least in part, in the nutrient medium 5 by means of a support (not shown); in the embodiment in Figure 10, the sensor 20 and/or the stress-imparting means 30 is/are disposed in a wall of the culture device, in this case in the thickness of the bottom wall 13 of the basket 3; in the embodiment in Figure 11, the sensor 20 and/or the stress-imparting means 30 is/are disposed under the bottom wall 13 of the basket 3; in the embodiment in Figure 12, the sensor 20 and/or the stress-imparting means 30 is/are disposed in a wall of the culture device, in this case in the thickness of the bottom wall 11 of the well 2; and in the embodiment in Figure 13, the sensor 20 and/or the stress-imparting means 30 is/are disposed on the bottom wall of the well 2.

Figure 14 shows the possibility of the detection system 10 including a sensor 20 and/or stress-imparting means 30 disposed, at least in part, under the bottom wall 11 of the well 2, and Figure 15 shows the possibility of said detection system being secured to a support 12, which can be fastened in optionally-removable manner on the culture system, coming to rest against the top wall of the basket 3, for example.

The examples in Figures 8 to 15 can be useful when the skin is exposed to light radiation, for example, and when the various thicknesses through which the light passes present optical properties that are compatible with the optical properties of the skin being measured through the thicknesses.

Thus, by way of example, the Figure 11 embodiment corresponds to a sensor or to a light source 30 placed below a bottom wall 13 having light-absorbing characteristics that are known.

In variants that are not shown, the detection system includes more than one sensor or stress-imparting means,
with each of the sensors or stress-imparting means being suitable, for example, for being disposed relative to the sample 4 in one of the above-mentioned configurations.

Naturally, the invention is not limited to the embodiments described above. In particular, sensors other than those described above can be used.

Where appropriate, all the wells of a common plate can be made in identical manner with identical sensors and/or stress-imparting means.

In a variant, at least two wells of a common plate can be made with sensors and/or stress-imparting means that are different.

The invention can be applied to reconstructed tissues other than skin.

Throughout the description, including in the claims, the expression "comprising a" should be understood as being synonymous with "comprising at least one", unless specified to the contrary.
CLAIMS
1. A device comprising:
   - a sample of reconstructed tissue; and
   - a detection system that is at least partially in contact with the tissue sample and/or with a device serving to culture said sample, said detection system being configured to measure the influence of a physical, biological, and/or chemical stress on at least a fraction of the sample, or vice-versa.

2. A device according to claim 1, wherein the reconstructed tissue is a reconstructed skin.

3. A device according to claim 1 or 2, wherein the detection system is configured to measure the influence of a physical, biological, and/or chemical stress on at least a fraction of the sample.

4. A device according to claim 1 or 2, wherein the detection system is configured to measure the influence of at least a fraction of the sample on a physical, biological, and/or chemical stress.

5. A device according to any preceding claim, wherein the detection system includes a sensor in contact with the sample.

6. A device according to any preceding claim, wherein the detection system includes a sensor in contact with the device serving to culture the tissue.

7. A device according to any preceding claim, wherein the detection system includes stress-imparting means in contact with the sample.

8. A device according to any preceding claim, wherein the detection system includes stress-imparting means in contact
with the device serving to culture the tissue.

9. A device according to any preceding claim, wherein the detection system includes a sensor for detecting amino acids, vitamins, acetylcholine, oxygenated water, and/or enzymes, in particular glucose dehydrogenase enzymes, glutamate dehydrogenase enzymes, and/or NADH oxidase enzymes.

10. A device according to any preceding claim, wherein the detection system includes a sensor for detecting partial pressure of at least one gas.

11. A device according to claim 10, wherein the sensor is a $P_{O_2}$, $P_{CO_2}$ sensor.

12. A device according to any preceding claim, wherein the detection system includes a biological-tissue sensor.

13. A device according to any preceding claim, wherein the detection system includes at least one electrode.

14. A device according to claim 13, wherein the electrode is in contact with the sample.

15. A device according to claim 14, wherein the electrode includes, at least on its surface, a material that is inert relative to the sample.

16. A device according to claim 15, wherein said material is a passivated material.

17. A device according to any preceding claim, wherein the detection system includes at least an optrode and/or an optical fiber and/or a bundle of optical fibers in contact with the sample.

18. A device according to any preceding claim, wherein the
detection system is arranged to image a fraction of the sample and/or to analyze and/or to locate a chemical or biological entity in the sample.

195. A device according to any preceding claim, wherein the detection system includes at least one optical sensor and light source and/or an optical sensor that is sensitive to ultraviolet radiation.

20. A device according to claim 19, wherein the source emits UVa radiation and/or UVb radiation.

21. A device according to claim 19 or 20, wherein the optical sensor is sensitive to UVa radiation and/or to UVb radiation.

22. A device according to any one of claims 1 to 21, wherein the detection system includes a light source enabling the sample to be exposed to light having a wavelength that is selected so as to excite a fluorescent marker, and a sensor that is sensitive to the wavelength of the light emitted by the fluorescent marker.

23. A device according to claim 21 or claim 22, wherein the optical sensor includes at least one of: a photodiode; a phototransistor; or a photoconductor.

24. A device according to any preceding claim, wherein the detection system includes a temperature sensor.

25. A device according to any preceding claim, wherein the detection system includes a laser.

26. A device according to any preceding claim, wherein the detection system includes a heating or cooling source enabling the sample to be heated and/or cooled.

27. A device according to any preceding claim, wherein the
detection system includes a sensor that is sensitive to the concentration of at least one ionic species.

28. A device according to claim 27, wherein the sensor is one or more pH sensor(s).

29. A device according to any preceding claim, wherein the sample is a sample of reconstructed skin.

30. A device according to claim 29, wherein the sample comprises an epidermis containing keratinocytes.

31. A device according to claim 29 or claim 30, wherein the sample includes a corneum.

32. A device according to claim 29 or 30, wherein the sample does not include a corneum.

33. A device according to any one of claims 29 to 32, wherein the sample contains melanocytes.

34. A device according to any one of claims 29 to 33, wherein the sample contains Langerhans cells.

35. A device according to any one of claims 29 to 34, wherein the sample comprises both a dermis and an epidermis.

36. A device according to claim 1, wherein at least part of the detection system is in contact with the sample.

37. A device according to any preceding claim, wherein at least part of the detection system is positioned above the sample.

38. A device according to claim 37, wherein the system includes a sensor and/or stress-imparting means positioned above the sample.
39. A device according to any preceding claim, wherein at least part of the detection system is positioned below the sample.

40. A device according to claim 39, wherein said system includes a sensor and/or stress-impacting means positioned below the sample.

41. A device according to claim 30, wherein at least part of the detection system is positioned inside the epidermis.

42. A device according to claim 41, wherein said system includes a sensor and/or stress-impacting means positioned, at least in part, inside the epidermis.

43. A device according to any preceding claim and to at least claim 35, wherein at least part of the detection system is positioned between the dermis and the epidermis.

44. A device according to claim 43, wherein said system includes a sensor and/or stress-impacting means positioned between the dermis and the epidermis.

45. A device according to any preceding claim and to at least claim 35, wherein at least part of the detection system is positioned inside the dermis.

46. A device according to claim 45, wherein the system includes a sensor positioned inside the dermis.

47. A device according to any preceding claim, wherein the sample presents a surface lying in the range about 0.3 cm² to about 1.35 cm².

48. A device according to claim 47, wherein said surface lies in the range about 0.38 cm² to about 1.12 cm².
49. A device according to any preceding claim, wherein the culture device includes a basket in which the sample is disposed.

50. A device according to claim 49, wherein the culture device includes a well to which the basket can be fastened in removable manner.

51. A device according to any preceding claim, wherein the detection system includes means for processing signals coming from at least one sensor.

52. A device according to any preceding claim, wherein the detection system includes an interface for connection to a micro-computer.

53. A method of measuring at least one physical, chemical, and/or biological parameter of at least one sample of tissue using a device as defined in any one of claims 1 to 52, wherein said method comprises:
   · exposing the sample to at least one physical, chemical, and/or biological stress; and
   · using the detection system to measure at least one physical, chemical, and/or biological parameter of the sample during and/or after said stress.

54. A method according to claim 53, wherein the at least one sample of tissue is of reconstructed skin.

55. A method of measuring the influence of at least one sample of tissue of a device as defined in any one of claims 1 to 52, on a physical, chemical, and/or biological stress parameter, wherein said method comprises:
   · disposing the sample in such a manner as to enable it to influence the physical, chemical, and/or biological stress; and
using the detection system to collect at least one item of information that is representative of the influence of the sample on said stress.

56. A method according to claim 55, wherein the at least one sample of tissue is of reconstructed skin.

57. A method according to any one of claims 53 to 56, wherein before the sample is exposed to the stress, the value of the parameter is measured.

58. A method according to any one of claims 53 to 57, wherein the parameter is the concentration of at least one chemical and/or biological entity.

59. A method according to the preceding claim, wherein the chemical and/or biological entity is selected from: amino acids; vitamins; acetylcholine; oxygenated water; O₂, CO₂, H⁺; and/or enzymes.

60. A method according to claim 59, wherein the enzyme is glucose dehydrogenase enzymes, glucamate dehydrogenase enzymes, and/or NadH oxidase enzymes.

61. A method according to anyone of claims 53 to 60, wherein the stress is physical stress.

62. A method according to the preceding claim, wherein the stress includes exposing the sample to light radiation.

63. A method according to the preceding claim, wherein the radiation comprises UVa radiation and/or UVb radiation.

64. A method according to the preceding claim, wherein it is possible to measure a magnitude that is representative of the absorption, by the sample, of at least a fraction of the spectrum of the light radiation.
65. A method according to any one of claims 53 to 64, wherein the stress includes applying a substance to the sample.

66. A method according to claim 65, wherein the substance is a cosmetic or a care product.

67. A method according to any one of claims 53 to 66, wherein the sample is exposed to thermal stress.

68. A method according to any one of claims 53 to 67, wherein the sample is exposed to mechanical stress.

69. A method according to any one of claims 53 to 68, wherein the physical stress includes at least one electrical stimulation.

70. A method according to any one of claims 53 to 69, wherein at least two measurements of said parameter are taken, separated by a time interval.

71. A method of manufacturing a device as defined in any one of claims 1 to 52, wherein at least part of the detection system serves as a support for culturing the reconstructed-tissue sample.

72. A method according to the preceding claim, wherein the detection system is present from the start of culturing the tissue.

73. A method according to claim 71, wherein the detection system is implanted in the sample while said sample is being cultured.

74. A method of manufacturing a device according to any one of claims 1 to 52, wherein at least part of the detection system is implanted in tissue that is completely
reconstructed and viable.

75. A method according to the preceding claim, wherein at least part of the detection system is implanted in the reconstructed tissue while said system is being used.

76. A method according to the preceding claim, wherein the detection system is implanted in the reconstructed tissue between two measurements.

77. A method of measuring at least one physical, chemical, and/or biological parameter of a sample of reconstructed tissue, in which the sample is subjected to a physical, chemical, and/or biological stress, and in which at least one measurement is taken of the parameter in the presence of the stress.
FIG.1  PRIOR ART

FIG.2

FIG.3
### A. CLASSIFICATION OF SUBJECT MATTER

C12N3/00  C12N5/06

According to International Patent Classification (IPC) or to both national classification and IPC

### B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12M  C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPOn-Internal, PAJ, WPI Data

### C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2001/043918 A1 (MASINI MICHAEL A ET AL) 22 November 2001 (2001-11-22) paragraphs [0025], [0026], [0033], [0034], [0049] - [0055]; figures 1-5</td>
<td>1-77</td>
</tr>
<tr>
<td>A</td>
<td>US 5 914 264 A (KORMAN ET AL) 22 June 1999 (1999-06-22) column 5, line 42 - line 46 column 6, line 21 - line 36; figure 1</td>
<td>1-77</td>
</tr>
</tbody>
</table>

X Further documents are listed in the continuation of Box C.

X See patent family annex.

* Special categories of cited documents:

  * **A** document defining the general state of the art which is not considered to be of particular relevance
  * **B** earlier document but published on or after the international filing date
  * **L** document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
  * **O** document referring to an oral disclosure, use, exhibition or other means
  * **P** document published prior to the international filing date but later than the priority date claimed
  * **T** later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
  * **X** document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
  * **Y** document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
  * **Z** document member of the same patent family

Date of the actual completion of the international search: 27 March 2006

Date of mailing of the international search report: 03/04/2006

Name and mailing address of the ISA/ European Patent Office, P.O. Box 5816 Patentlaan 2 NL-2280 HV Rijswijk Tel. +31-70-940-2040, Tx. 31 651 epo nl, Fax +31-70-940-3016

Authorized officer: Cubas Alcaraz, J
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patent document cited in search report</td>
<td>Publication date</td>
<td>Patent family member(s)</td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>-----------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>US 2001043918 A1</td>
<td>22-11-2001</td>
<td>NONE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 4691696 A</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2209013 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0807164 A2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>JP 10511851 T</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WO 9620999 A1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 5686303 A</td>
</tr>
</tbody>
</table>