CANDIDATE'S ANSWER

A, EQE 2019

1. A device for culturing cells, comprising:
a frame (1);
a first gas-permeable liquid-impermeable membrane (2a) and a second gas-permeable liquid-impermeable membrane (2b),
wherein the first and second membranes (2a, 2b) are attached to opposite sides of the frame (1) by means of a leak-proof seal in order to form a cell chamber (4) between the first and second opposing membranes (2a, 2b) and the frame (1), and wherein the device further comprises a leak-proof opening, the leak-proof opening being provided by the frame (1) comprising at least one leak-proof resealable aperture (3a, 3b), and/or at least one of the first and second membranes (2a, 2b) being resealably attached to the frame (1) in a leak-proof manner by means of a pressure sensitive adhesive.

2. The device as claimed in claim 1, wherein at least one of the first and second membranes (2a, 2b) is optically transparent.

3. The device as claimed in claim 1 or 2, wherein at least one of the first and second membranes (2a, 2b) comprises polyethylene.

4. The device as claimed in any preceding claim, wherein at least one of the first and second membranes (2a, 2b) has a gas permeability performance of from 1 x 10^-16 to 3 x 10^-16 m^3/(s.Pa) for oxygen, O2, and from 6 x 10^-16 to 7 x 10^-16 m^3/(s.Pa) for carbon dioxide, CO2, when measured at a pressure of 10^5 Pa and a temperature of 37degC.

5. The device as claimed in any preceding claim, wherein the inner surface of at least one of the first and second membranes (2a, 2b) facing the cell chamber (4) is coated with a substance that facilitates cell adhesion.

6. The device as claimed in any preceding claim, wherein the first membrane (2a) is separated from the second membrane (2b) by an average distance of from 1 mm to 5 mm.

7. The device as claimed in any preceding claim, wherein the leak-proof opening is provided by the frame (1) comprising the at least one leak proof resealable aperture (3a, 3b), and the leak-proof resealable aperture (3a, 3b) is in the form of a gasket, optionally the gasket comprises an elastomeric material.

8. The device as claimed in claim 7, wherein the gasket comprises an elastomeric material together with an antimicrobial agent integrated therein.

9. The device as claimed in any preceding claim, wherein the leak-proof opening is provided by the frame (1) comprising two or more leak-proof resealable apertures (3a, 3b).

10. The device as claimed in any preceding claim, wherein the leak-proof opening is provided only by the frame (1) comprising at least one leak-proof resealable aperture (3a, 3b), and wherein the first and second membrane (2a, 2b) are secured to the frame (1) by ultrasonic welding.

11. Use of a device as claimed in any of claims 1 to 10, in a method of culturing cells, comprising:
(a) suspending the cells to be cultured in a cell culture medium to form a cell suspension;
(b) introducing the cell suspension in the device; and
(c) incubating the device containing the cell suspension in conditions allowing cell growth.

12. The use as claimed in claim 11, wherein introducing the cell suspension into the device comprises completely filling the cell culture chamber (4) of the device with the cell suspension such that there is no head space containing air in the cell culture chamber (4).

13. A system comprising:
a device for culturing cells as claimed in any of claims 1 to 10; and
a rack arranged to hold the device.

14. A method of manufacturing a device as claimed in any of claims 1 to 10, the method comprising:

providing a frame (1), a first gas permeable liquid impermeable membrane (2a), and a second gas permeable liquid impermeable membrane (2b);

attaching the first and second membranes (2a, 2b) to opposite sides of the frame (1) by means of a leak-proof seal in order to form a cell chamber (4) between the first and second opposing membranes (2a, 2b) and the frame (1); and

providing a leak-proof opening for the device, the leak-proof opening being provided by the frame (1) comprising at least one leak-proof resealable aperture (3a, 3b), and/or at least one of the first and second membranes (2a, 2b) being resealably attached to the frame (1) in a leak-proof manner by means of a pressure sensitive adhesive.

15. A method as claimed in claim 14, wherein the leak-proof opening is provided only by the frame (1) comprising at least one-leak proof aperture (3a, 3b), and wherein the method comprises securing each of the first and second membranes (2a, 2b) to the frame (1) by ultrasonic welding.

The present invention concerns a device for culturing cells, use of the device in a method of culturing cells, a system comprising the device and a method of manufacturing the device.

Cell culture refers to the removal of cells from their natural environment and their subsequent growth in an artificial environment. To successfully grow cells in culture the cells need to be supplied with the essentials for growth and respiration. The cells are usually grown under controlled conditions including pH, temperature and exchange of gases such as oxygen (O2) and carbon dioxide (CO2). Devices for culturing cells (i.e. cell culture devices) are usually kept in an incubator in which

oxygen is supplied at about 20% concentration, to ensure that the cells can obtain sufficient oxygen for adequate growth.

D1 discloses an existing form of cell culture device. The device is in the form of a muilti-well plate that includes a flat surface comprising a series of wells which hold the cell culture medium and cells. The plate is covered by a lid.

D1 discloses that the plate is formed of a rigid air tight plastic that is optically transparent. The lid in D1 can be made of a gas-permeable, liquid impermeable membrane sold under the trade name Gas Easy (TM).

D2 discloses the Gas Easy (TM) membrane. D2 also discloses that the membrane is used to leak-proof seal multi-well plates by applying the membrane over the surface of the plate such that an adhesive applied to the membrane is able to seal around the edge of each well under the application of pressure.

D2 also discloses that more than one membrane may be provided by being juxtaposed next to each other. This means that two or more membranes are arranged side by side so as to cover a large multi-well plate.

In D1 and D2 only the upper surface of the well plates are disclosed as being covered with a gas-permeable membrane. This means that the supply of oxygen only comes from the space in the device above the surface of the cell culture medium, known as the head space. Thus the surface provided for gas exchange is limited and may result in low rates of cell growth. Further, there may be a sharp initial drop in pH within the first hour or two caused by CO2 from the head space dissolving in the medium. This drop in pH can negatively affect the rate of cell growth.

It is an object of the present invention to overcome this problem of the prior art by providing a device for culturing cells that is able to increase the cell growth rate.

According to a first aspect of the invention, there is provided a device for culturing cells as set out in accompanying claim 1. Significantly, the device comprises first and

second gas-permeable liquid-impermeable membranes. “gas-permeable” means that the membranes contain pores that allow gases to pass through them. “liquid-impermeable” means that liquids are not able to pass through the membranes. Significantly still, the first and second membranes are attached to opposite sides of the frame by means of a leak-proof seal in order to form a cell chamber between the first and second opposing membranes and the frame.

D1 only discloses that one gas-permeable, liquid-impermeable membrane is provided in the form of a lid that covers the cell chamber. Therefore, in D1 the cell-culture chamber is formed between the gas-permeable membrane and the rigid, air-tight plastic plate.

D2 only discloses that the multi-well plates may be covered by gas-permeable liquid impermeable membranes. While D2 does disclose that more than one membrane can be provided, the membranes are juxtaposed next to one another and are not attached to opposite sides of the frame. They are instead all attached to the same side of the frame. Consequently, in D2 a cell chamber is not formed between two opposing membranes and the frame as claimed.

Advantageously, the claimed solution means that the surface area provided for gas-exchange is increased by the provision of the two gas-permeable membranes on opposite sides of the frame. As a result, the problems of D1 and D2 as mentioned above are overcome, by increasing the exchange of gases, in particular CO2 and O2. As a result of the increased exchange of gases, the cell growth rate is increased.

Preferably, the device comprises the features of claim 2. An optically transparent membrane advantageously permits observations of the cells. The membrane may comprise polymers such as polyethylene, polycarbonate, polypropylene or a silicone copolymer. The choice of polymer will depend on the type of cell to be grown, the rate of gas transfer and optical transparency.

Preferably, the device comprises the features of claim 3, which provide a good combination of features for cell culture.

Preferably, the device comprises the features of claim 4, which result in excellent rates of cell growth.

Preferably, the device comprises the features of claim 5. Advantageously, this enables the device to be used with adherent cells that require attachment to a surface in order to grow. The coating may include molecules such as gelatine, collagen, and fibronectin.

Preferably, the device comprises the features of claim 6. The spacing provides an optimum amount of space for the cells to grow and still have sufficient gas exposure.

The frame may be made from a biocompatible composition that comprises a plastic or thermoplastic.

The device may be 10 cm to 15 cm long, 7 cm to 9 cm wide, 0.2 cm to 2 cm high. The membranes may each have a thickness of 0.05 mm to 0.15 mm.

Preferably, the device comprises the features of claim 7. The gasket is a type of mechanical seal. Advantageously, when a needle tip is inserted into the gasket, it closes around and engages the tip to form a leak-proof seal, and reseals itself after the tip is withdrawn.

Preferably, the gasket comprises an elastomeric material. Elastomeric means that the material can deform and the spring back. The elastomeric material may be natural, such as natural rubber, or synthetic such as silicone rubber or fluorocarbon rubber.

Preferably, the device comprises the features of claim 8. The antimicrobial agent beneficially stops the aperture from being a potential route for infection of the cell culture by microbes in the surrounding area. The antimicrobial agent may be trictosan or chloroxylenol.

Preferably, the device comprises the features of claim 9. Advantageously, one of the apertures can be used to introduce substances into the cell chamber while other substances are removed via the other aperture.

Preferably, each aperture has a diameter, generally from 1 mm to 2 mm, sufficient for standard needles to pass through.

Preferably, the device comprises the features of claim 10. In this example, only at least one aperture is provided to form the leak-proof opening. The membranes are not resealably attached to the frame. The membranes are secured to the frame by ultrasonic welding, i.e. melting the membrane to the frame in a manner that results in a leak-proof seal between the membrane and the frame. In another example, an adhesive such as a hot melt adhesive may be used.

According to a second aspect of the invention, there is provided the use of the device in a method of culturing cells as claimed in claim 11.

Preferably, the use of the device comprises the features of claim 12. Advantageously, completely filling the cell culture chamber with cell suspension such that there is no head space containing air prevents a drop in pH caused by dissolved CO2 from the headspace. This increases the cell growth rate. Moreover, the device can be tilted or gently shaken without causing formation of foam which may disrupt the cell growth.

According to a third aspect of the invention, there is provide a system as claimed in claim 13. The rack can hold the device in the incubator so that there is sufficient space between each membrane and the incubator to allow air to circulate. This means that the first and second membranes have direct contact to air in order to allow gas exchange. The gas exchange rate, and therefore the cell growth is increased.

According to a fourth aspect of the invention there is provided a method of manufacturing a device as claimed in claim 14.

Preferably, the method comprises the features of claim 15.

Examination Committee I: Paper A - Marking Details - Candidate No

Category		Max. possible	Marks Marker 1	Marker 2
Claims	Independent product claim	42	42	42
Claims	Independent method claim	15	13	13
Claims	Dependent claims	27	26	25
Description	Description	16	12	12
Total			**93**	**92**

Examination Committee I agrees on 93 points and recommends the grade PASS