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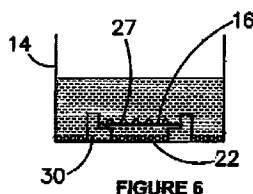
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(54) Title: CELL CULTURE DEVICE AND METHOD



(57) Abstract: A cell culture device (10) including a base (12) and a perimeter wall (14) extending upwardly from the base (12) to define a vessel for holding a cell culture medium therein. Means (30) for mounting a cell culture surface (16) within the vessel above and spaced from the base (12) are provided. The mounting (30) is formed integrally with the base (12).

WO 2011/044622 A1

Cell culture device and method

Field of the invention

The present invention relates generally to cell culture and more particularly to cell culture apparatus and methods for cell culture.

5 Background of the invention

Cell culture devices are generally dishes, plates or flasks that are used for the maintenance and or expansion of eukaryotic cells *in vitro*. In use, these devices generally contain one or more cell lines or types, together with a medium that provides essential ingredients for cell growth and/or homeostasis.

- 10 Some cells (referred to herein as 'target cells') require conditioned medium for culture. Conditioning of medium is generally provided by culturing a target cell with one or more further cell types or lines. The latter are sometimes call 'feeder cells' and the resulting culture may be known as a 'co- culture'.

- One difficulty with co-cultures is that it can be difficult to stop the feeder cells from
15 growing into the populations of target cells.

Another problem is that certain target cells, when co-cultured, tend to have a very short life span, especially when cultured at low density, the latter being particularly desirable for testing of specific target cell types.

- A further problem is that it is difficult to observe growth or response of target cells by
20 light microscopy in a co-culture because the feeder cells in the co-culture tend to grow to confluence across the bottom of the cell culture device and this tends to obstruct observation of the target cells.

Also, it can be quite difficult to simply recover target cells from a co-culture system.

It is an object of the invention to provide an improved cell culture apparatus and method that at least in part alleviates some of the problems identified above.

Reference to any prior art in the specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common
5 general knowledge in Australia or any other jurisdiction or that this prior art could reasonably be expected to be ascertained, understood and regarded as relevant by a person skilled in the art.

Summary of the invention

In one aspect the invention provides a cell culture device including:

- 10 a base;
- a perimeter wall extending upwardly from the base to define a vessel for holding a cell culture medium therein;
- means for mounting a cell culture surface within the vessel above and spaced from the base;
- 15 wherein the mounting is formed integrally with the base.

Advantageously, the base includes one or more cell culture regions for culture of a cell therein. Preferably, the one or more cell culture regions are each defined by the application of a surface coating agent to the base in those region/s.

Advantageously, the base of the cell culture device is generally circular and the cell
20 culture region is a ring near or at the perimeter of the base.

In preferred embodiments, the cell culture surface is a cover slip or similar substrate. The cover slip is preferably coated with a surface coating agent to permit the adherence of cells.

The means for mounting the cell culture surface preferably take the form of one or more upright members integrally formed with the base during manufacture. The members are preferably located spaced from the perimeter wall. Advantageously, the one or more cell culture regions are sufficiently spaced from the upright members such that the cell culture regions do not underlie the cell culture surface.

Preferably, the upright members are of a height sufficient to space the cell culture surface from the base and enable passage of the cell culture medium between the base and the cell culture surface. The height of the upright members is also such that the cell culture surface can be immersed in the cell culture medium in the vessel.

- 10 Preferably, the upright members are adapted for detachably securing the cell culture surface. The ends of the upright members distal from the base are preferably formed with a recess or shoulder formation on which the cell culture surface is located.

In another aspect the invention provides a cell culture device including:

- a base;
- 15 a perimeter wall extending upwardly from the base to define a vessel for holding a cell culture medium therein;
- wherein the base includes one or more cell culture regions for culture of a cell therein;
- at least a further region for supporting a cell culture surface thereon; and
- 20 wherein the cell culture regions and the further region do not overlap.

Preferably, the one or more cell culture regions are each defined by the application of a surface coating agent to the base in those region/s.

Advantageously, the base of the cell culture device is generally circular and the cell culture region is a ring near or at the perimeter of the base. The further region for supporting a cell culture surface thereon is preferably the central region of the base.

In preferred embodiments, the cell culture surface is a cover slip or similar substrate.

- 5 The cover slip is dimensioned to be received in the cell culture device in the central region of the base and preferably equidistant from the perimeter so as to not overlie the one or more cell culture regions.

The cover slip is preferably coated with a surface coating agent to permit the adherence of cells.

- 10 The cover slip is preferably supported on the base of the cell culture device by capillary action. A small droplet of water may be placed in the centre of the base and the cover slip placed on top.

Alternatively, in a preferred embodiment, the further region for supporting a cell culture surface thereon is above and spaced from the base of the cell culture device.

- 15 In this embodiment, the further region is defined by one or more upright members integrally formed with the base during manufacture. Preferably the cell culture surface is a cover slip or similar substrate and the cover slip is coated with a surface coating agent to permit the adherence of cells.

Brief description of the figures

- 20 The invention will now be described, by way of non-limiting example, with reference to the accompanying figures in which:

Figure 1 is a cross-sectional view of a cell culture device according to a first embodiment of the invention;

Figure 2 is a plan view of the cell culture device illustrated in Figure 1;

Figure 3 is a cross-sectional view of a cell culture device according to a second embodiment of the invention;

Figure 4 is a plan view of the cell culture device illustrated in Figure 3;

Figure 5 is a perspective view of cover slip mounts in accordance with the second
5 embodiment of the invention; and

Figure 6 is a cross-sectional view illustrating primary hippocampal neurons being cultured in the cell culture device according to an embodiment of the invention,

Detailed description of the embodiments

Referring to Figures 1 and 2 there is illustrated a cell culture device in the form of a cell
10 culture dish 10 having a base 12 and a perimeter wall 14 extending upwardly from the base of the dish 10 and defining a vessel. Preferably the dish is circular but other shapes are envisaged. In alternate embodiments (not illustrated), the dish 10 may instead be referred to as a well, and may be one of several wells formed in a culture plate.

15 In a first embodiment of the invention a droplet of water is placed in the centre of the base 12 of the dish 10 and a cell culture surface in the form of a cover slip 16 is placed on the droplet. The cover slip 16 is located equidistant from the perimeter wall 14 and is held on the base 12 by capillary action.

A surface coating 18 is applied to the base 12 of the dish 10 to form an adherent
20 surface for the culture of a cell therein. The surface coating 18 is advantageously applied in a ring 20 around the perimeter of the base 12 as illustrated to form a cell culture region 22, leaving the central region 24 of the base 12 free from cell cultures.

A surface coating 26 is also applied to the upper surface of the cover slip 16 to permit
25 the adherence of cells. Poly-L-lysine, poly-D-ornithine, gelatin, and collagen are examples of surface coatings. Others are known in the art.

In one embodiment, particular where cell lines are to be cultured that secrete an extra cellular matrix, a surface coating agent is not required.

After washing, cells 27 are placed on the cover slip 16 and the cell culture region 20 and a cell culture or growth medium 28 is added to the dish 10 to facilitate the cell growth.

- 5 Since the cover slip 16 and the cell culture region 20 do not overlap, the cover slip 16 may be illuminated from beneath the dish 10 and the cells 27 may be easily visually assessed, for example by light microscopy. At the conclusion of the process, the cover slip 16 is removed from the dish 10 using forceps (not shown).

- 10 In a second embodiment of the invention illustrated in Figures 3 to 5, the cell culture dish 10 is manufactured with integral upright members 30. The Figures show three upright members 30 integrally formed with the base 12 but alternate numbers of upright members 30 may be used. It is also envisaged that support members for the cell culture surface or cover slip 16 may be provided in different configurations or locations within the dish 10. For example, a pair of upright members 30 may be provided on the base
15 12, a plurality of upright members 30 may be provided in a half-moon shape on the base 12, or alternatively supporting struts (not shown) may extend inwardly from the perimeter wall 14.

- In the embodiment illustrated, upright members 30 are located spaced from the perimeter wall 14 and provide support for cover slip 16. The height of the upright
20 members 30 is such that the cover slip 16 is adequately spaced from the base 12 of the dish 10 to enable free flow of a cell culture medium 28 between the cover slip 16 and the base 12, while still remaining within the vessel so that it can be fully immersed in the growth medium 28.

- In this embodiment, upright members 30 are placed circumferentially in the central
25 region of the base 12 thereby providing sufficient clearance for the application of a surface coating 18 to the base 12 of the dish 10 to form an adherent surface for the culture of a cell therein. The surface coating 18 is advantageously applied in a ring 20

around the perimeter of the base 12 as illustrated to form a cell culture region 22, leaving the central region 24 of the base 12 free from cell cultures.

A surface coating 26 is also applied to the upper surface of the cover slip 16 to permit the adherence of cells.

- 5 As illustrated in Figure 5 it is preferred that the upright members 30 are able to securely hold the cover slip 16 in place particularly once the cover slip 16 has been immersed in the growth medium 28. To facilitate this, each of the ends 32 of the upright members 30 are formed with a shoulder 34. Each shoulder 34 is inwardly facing so that a confined support surface is provided for the cover slip 16. Alternate configurations of the ends 32
10 of the upright members 30 are also envisaged.

After washing, cells 27 are placed on the cover slip 16 and the cell culture region 20 and a cell culture or growth medium 28 is added to the dish 10 to facilitate the cell growth.

- As in the first embodiment, since the cover slip 16 and the cell culture region 20 do not overlap, the cover slip 16 may be illuminated from beneath the dish 10 and the cells 27
15 may be easily visually assessed. At the conclusion of the process, the cover slip 16 is easily lifted off the upright members 30 using forceps (not shown).

Example

- Primary hippocampal neurons were cultured in the cell culture device 10 (Figure 6) on top of a coverslip that was coated with poly-D-lysine to allow cell to adhere. Cortical
20 neurons were cultured at the bottom of the well to provide nutritional support. After 20 days in culture, the cells were fixed with paraformaldehyde, the coverslip transferred to a staining chamber and stained with antibodies to neuronal proteins (beta-3-tubulin, tau1, MAP2 and Drebrin). This revealed the complete maturation of the neurons and confirmed their viability.

- 25 It will be appreciated that the cell culture device and method is suitable to culture primary neurons under condition for long-term survival and full maturation.

It will be understood that the invention disclosed and defined in this specification extends to all alternative combinations of two or more of the individual features mentioned or evident from the text or drawings. All of these different combinations constitute various alternative aspects of the invention.

The claims defining the invention are as follows:

1. A cell culture device including:
 - a base;
 - a perimeter wall extending upwardly from the base to define a vessel for holding
 - 5 a cell culture medium therein;
 - means for mounting a cell culture surface within the vessel above and spaced from the base;
 - wherein the mounting is formed integrally with the base.
2. The cell culture device of claim 1 wherein the base includes one or more cell
- 10 culture regions for culture of a cell therein.
3. The cell culture device of claim 1 or 2 wherein the one or more cell culture regions are each defined by the application of a surface coating agent to the base in those region/s.
4. The cell culture device of any one of claims 1 to 3 wherein the base of the cell
- 15 culture device is generally circular.
5. The cell culture device of any one of claims 1 to 4 wherein the cell culture region is a ring near or at the perimeter of the base.
6. The cell culture device of claim 5 wherein the cell culture surface is a cover slip or similar substrate.
- 20 7. The cell culture device of claim 6 wherein the cover slip is coated with a surface coating agent to permit the adherence of cells.

8. The cell culture device of any one of claims 1 to 7 wherein the means for mounting the cell culture surface take the form of one or more upright members integrally formed with the base during manufacture.

9. The cell culture device of any one of claims 1 to 8 wherein the ends of the upright
5 members distal from the base are formed with a recess or shoulder formation on which the cell culture surface may be located.

10. A cell culture device including:

a base; and

a perimeter wall extending upwardly from the base to define a vessel for holding
10 a cell culture medium therein;

wherein the base includes one or more cell culture regions for culture of a cell therein;

at least a further region for supporting a cell culture surface thereon; and

wherein the cell culture regions and the further region do not overlap.

11. The cell culture device of claim 10 wherein the one or more cell culture regions
15 are each defined by the application of a surface coating agent to the base in those region/s.

12. The cell culture device of claim 10 or 11 wherein the base of the cell culture
20 device is generally circular and the cell culture region is a ring near or at the perimeter of the base.

13. The cell culture device of any one of claims 10 to 12 wherein the further region for supporting a cell culture surface thereon is the central region of the base.

14. The cell culture device of any one of claims 10 to 13 wherein the cell culture surface is a cover slip or similar substrate.
15. The cell culture device of claim 14 wherein the cover slip is dimensioned to be received in the cell culture device in the central region of the base and preferably
5 equidistant from the perimeter so as to not overlie the one or more cell culture regions.
16. The cell culture device of any one of claims 10 to 15 wherein the cover slip is supported on the base of the cell culture device by capillary action.
17. The cell culture device of any one of claims 10 to 16 wherein the cover slip is coated with a surface coating agent to permit the adherence of cells.
- 10 18. The cell culture device of any one of claims 10 to 12 wherein the further region for supporting a cell culture surface thereon is above and spaced from the base of the cell culture device.
19. The cell culture device of claim 18, wherein the further region is defined by one or more upright members integrally formed with the base during manufacture.
- 15 20. The cell culture device of claim 19 wherein the cell culture surface is a cover slip or similar substrate.
21. The cell culture device of claim 20 wherein the cover slip is coated with a surface coating agent to permit the adherence of cells.

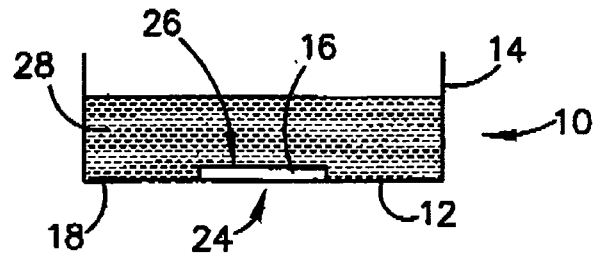


FIGURE 1

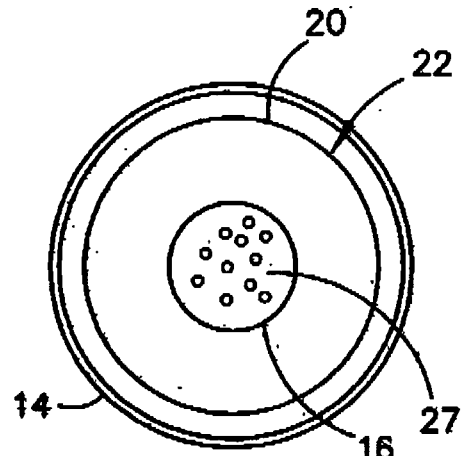


FIGURE 2

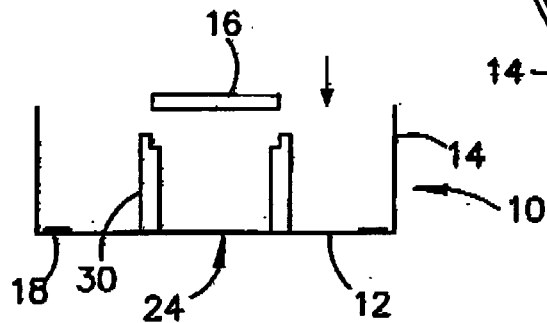


FIGURE 3

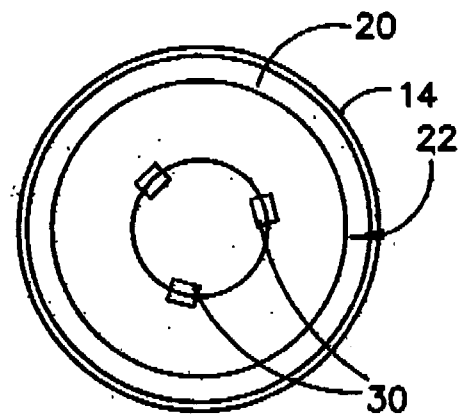


FIGURE 4

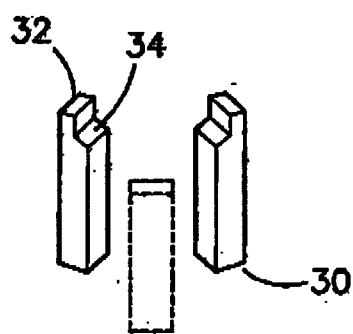


FIGURE 5

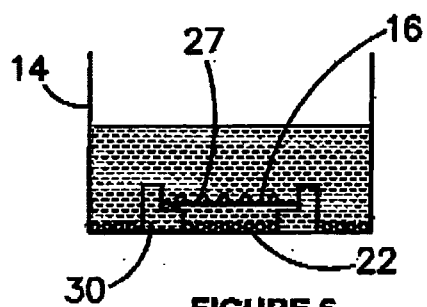


FIGURE 6

INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

C12M 1/00 (2006.01)

C12M 3/00 (2006.01)

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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)

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 practicable, search terms used)

EPODOC, WPI: IPC & ECLA Classmarks C12 M1/low, C12M 3/low, C12N 5/low. Keywords including; cell, tissue, culture, grow, dish, vessel, plate, separate, division, mount, protrusion, base, upright, stand.

PUBMED, using similar keywords.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
<u>X</u> A	FATH, T. et al., 'Primary support cultures of hippocampal and substantia nigra neurons', Nature Protocols, 2009, Vol.4, No.1, pages 78-85 [published online 18 December 2008] [Retrieved on 30 June 2010] Retrieved from the Internet <URL: http://www.nature.com/nprot/journal/v4/n1/full/nprot.2008.199.html#B15 > Figure 2 and caption; Procedure, step 2.	<u>10-17</u> 1-9, 18-21
X	US 4358539 A (BITTINGS) 9 November 1982 Abstract; Figure; Column 2, lines 10-15, 33-42, 60-65.	1, 2, 4, 8

☐ Further documents are listed in the continuation of Box C☒ See patent family annex

* Special categories of cited documents:	
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/AU2010/001347

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
US 4358539	NONE
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.	
END OF ANNEX	