

(12) PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 199856706 B2**
(10) Patent No. **732565**

(54) Title
cell-culture system

(51)⁶ International Patent Classification(s)
C12M 001/22 C12M 001/14

(21) Application No: **199856706** (22) Application Date: **1998 .01 .14**

(87) WIPO No: **WO98/31783**

(30) Priority Data

(31) Number	(32) Date	(33) Country
9700840	1997 .01 .16	GB

(43) Publication Date : **1998 .08 .07**

(43) Publication Journal Date : **1998 .10 .08**

(44) Accepted Journal Date : **2001 .04 .26**

(71) Applicant(s)
Smith and Nephew, PLC

(72) Inventor(s)
Patrick Lewis Blott; Dawn Poole

(74) Agent/Attorney
DAVIES COLLISON CAVE,1 Little Collins Street,MELBOURNE VIC 3000

(56) Related Art
GB 2268187
US 4748124
US 4715911

OPI DATE 07/08/98 APPLN. ID 56706/98
AOJP DATE 08/10/98 PCT NUMBER PCT/GB98/00116



AU9856706

T)

<p>(51) International Patent Classification ⁶ : C12M 1/22, 1/14</p>	<p>A1</p>	<p>(11) International Publication Number: WO 98/31783 (43) International Publication Date: 23 July 1998 (23.07.98)</p>
<p>(21) International Application Number: PCT/GB98/00116 (22) International Filing Date: 14 January 1998 (14.01.98) (30) Priority Data: 9700840.3 16 January 1997 (16.01.97) GB (71) Applicant (for all designated States except US): SMITH & NEPHEW, PLC [GB/GB]; 2 Temple Place, Victoria Embankment, London WC2R 2BP (GB). Heron House 15 Adam Street, London WC2N 6BA GB (72) Inventors; and (75) Inventors/Applicants (for US only): BLOTT, Patrick, Lewis [GB/GB]; 3 Newland Park Close, York YO1 3HW (GB). POOLE, Dawn [GB/GB]; 59 Belhouse Way, York YO2 3LL (GB). (74) Agent: SMITH & NEPHEW GROUP RESEARCH CENTRE; Group Patents & Trade Marks Dept., York Science Park, Heslington, York YO1 5DF (GB).</p>		<p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>
<p>(54) Title: CELL-CULTURE SYSTEM</p>		
<p>(57) Abstract A cell culture substrate device comprising a frame and a cell culture substrate material is disclosed. The frame is adapted to releasably secure the substrate material in preferably a flat configuration for the attachment of cells. The substrate material may further comprise a support layer. The device may be placed within a container, e.g. Petri-dish.</p>		



CELL-CULTURE SYSTEM

The present invention relates to substrates and vessels used for cell-culture techniques. In particular it relates to substrates on which cells may be
5 grown for subsequent transfer to a patient requiring a layer of cells.

WO 91/13638 describes a wound dressing which comprises a layer of polymeric material having a layer of cultured mammalian cells anchored to one surface. Such a dressing is suitable for use in the treatment of partial-thickness
10 wounds such as burns and skin donor sites. The cells are preferably epithelial cells which may be cultured from autologous skin cells taken by biopsy or may be from other sources. The cells are cultured and grown in the presence of the polymer substrate to which they attach. When the substrate is applied to a prepared site on the patient the cells transfer from the substrate to the patient
15 and continue to multiply to form a layer of epidermal cells. The dressings described are made by culturing the cells in contact with the surface of the polymeric dressing substrate, removing the substrate from the culture medium, and then placing the dressing on the patient such that the cells contact the wound-bed on the patient.

An object of the present invention is to provide an improved form of cell culture substrate device which is convenient to use in the culture and anchorage of cells to the substrate and subsequent transfer of cells to the patient. It is a further object to provide a cell culture system which also
20 achieves the above-mentioned objective.

According to the invention, there is provided a cell culture substrate device comprising a frame member formed from a material which has no or low toxicity cells and a cell culture substrate which is not toxic to cells and having at least one surface to which cells may attach, wherein said frame member is adapted to releasably secure said culture substrate in a suitable configuration of



the culture substrate for attachment of cells to said at least one surface and wherein the frame member further comprises upstanding walls extending around the said surface to which cells may attach, wherein the walls further comprise means for retaining the frame member within a container.

5

Further according to the invention, there is provided a cell culture system comprises a container and a cell culture substrate device, the device comprising a frame member formed from a material which has no or low toxicity cells and a cell culture substrate which is not toxic to cells and having at least one surface
10 to which cells may attach, said frame member being adapted to releasably secure said cell culture substrate in a suitable configuration of the culture substrate for the attachment of cells to said surface, wherein said frame member is of a shape and size to fit into said container, and said frame member has means for being retained within the container, such that said cell culture
15 substrate is held substantially parallel to one internal surface of said container. Preferably, the cell culture substrate device is as described in the immediately preceding paragraph.

The invention also extends to the cell culture substrate device together
20 with a batch of mammalian cells.

For convenience, the frame member of the cell culture substrate device will hereafter in this description be referred to as a "frame".



The frame is preferably of a suitable shape and size to fit into a cell-culture vessel. Preferred forms are generally square, rectangular or round although other shapes may be equally suitable.

- 5 The frame is adapted to secure a cell culture substrate in a suitable configuration for the attachment of cells to a surface thereof. The cell culture substrate preferably comprises a polymeric film which has a surface suitable for the attachment of cells. The film may be either hydrophilic or hydrophobic. The substrate may
10 comprise more than one layer of material.

- Suitable cell culture substrate materials include those mentioned in WO91/13638, i.e. polyesters, polypropylene, blends or copolymers of vinyl acetate, e.g. ethylene - vinyl acetate copolymer,
15 polyvinylidene chloride, polystyrene, polybutadienes, polyethylene, ionomers, e.g. SURLYN (trade mark), or copolymers and blends containing these compounds.

- Alternatively hydrophilic polymers such as hydrophilic
20 polyurethanes may form the culture substrate. The culture substrate has a first surface to which cultured mammalian cells may attach. This surface may be treated to modify its properties such as by coating with another material such as a collagen-based material, or by other means, e.g. corona-discharge or plasma treatment. The
25 substrate material is preferably a film of polymeric material. Preferably the substrate material is relatively conformable to body contours. The substrate may be continuous or may be apertured.

- The cell culture substrate material is preferably suitable for
30 application to a wound to serve as a wound dressing. Therefore the cell culture substrate is preferably conformable and elastomeric so as to conform to the contours of the wound to which it is applied. The material may be breathable. When the cell culture substrate has epithelial cells attached to a surface thereof, it may be applied
35 to a wound such that the cells are in contact with the wound to effect transfer of cells from the substrate to the wound to promote re-epithelialisation of the wound.

When the substrate comprises a planar material such as a polymeric film, the frame is preferably adapted to secure the substrate in a substantially flat configuration. In this way pooling of cells on the substrate may be minimised so that cells can be relatively evenly distributed upon the surface to which they attach. Therefore the frame preferably comprises a planar portion which engages at least a part of said cell culture substrate to releasably secure the substrate in a flat configuration in a plane which is parallel to said planar portion. The planar portion may comprise an open framework or a continuous layer of material. In a preferred form the planar portion comprises an open frame comprising a peripheral i.e. enclosed band, frame to which the cell culture substrate may be attached.

The cell culture substrate may be releasably secured to the frame e.g. by adhesive bonding, lamination, heat or radio-frequency welding or mechanical means such as clips. The bond between the frame and the cell culture substrate is preferably liquid-tight. Alternatively the frame may serve to press against part or all of the substrate to maintain it in a flat configuration against another surface which may be the inner surface of a container or cell culture vessel.

The upstanding walls of the frame may or may not extend continuously around the frame. The recess to act as the retaining means which may engage the edge of a container such as a petri dish or cell culture vessel. Preferably the walls are integral with the other parts of the frame. When the walls are continuous and surround the preferred planar portion of the frame, and the cell culture substrate is bonded to the frame, the frame and substrate may, together, form a container in which cells may be cultured.

The frame is made from materials which are non-toxic or not significantly toxic to cells. Suitable materials include polyesters (e.g. polyethylene terephthalate, polyethylene naphthalate), including blends and copolymers thereof, polyolefins, polystyrene.



polycarbonate, acrylic materials, polyetherimides, ethylene-vinyl acetate copolymers and blends and copolymers containing any of these or other suitable materials. The frame may be formed by moulding e.g. blow moulding thermoforming, vacuum or pressure forming or injection moulding. The frame
5 may be formed from a film of polymer by vacuum forming and heating to form the desired shape.

The culture substrate may further comprise a support layer. The culture substrate may be releasably attached to the support layer over a part or all of
10 one of its surfaces. Preferably the surface to which a support layer is attached is opposed to the surface to which cells attach. In this form, the culture substrate and support layer are secured in a flat configuration by the frame. The support layer preferably comprises a layer of a polymeric film material which is stiffer than the culture substrate and thus it helps to maintain the
15 culture substrate flat and free of wrinkles. The support layer may comprise a film of a material similar to those which are suitable to form the frame. The support layer is preferably releasably attached over the entire surface of the rest of the culture substrate which is opposed to the surface to which cells attach. The support is preferably attached in such a way that it may be removed from
20 the rest of the culture substrate by peeling when required.

In a preferred form of the invention, the cell culture substrate material comprises a first surface to which cultured mammalian cells may attach and a second opposed surface which is releasably attached over at least the greater part of its
25 second surface to the first surface of a continuous polymeric film which forms the support layer of the culture substrate.

In one embodiment the second surface of the cell culture is releasably attached to a portion, preferably central portion, of the support layer.
30

The remaining part of the support layer not in contact with the second opposed surface of the substrate material may be releasably or fixedly attached



to the frame.

Embodiments of a cell culture substrate device according to the invention will now be described, by way of example only, with reference to the
5 accompanying drawings which are :-

Fig. 1, a perspective view of a device according to the invention;

Fig. 2, a cross sectional view of the device shown in Fig 1; placed within
10 a vessel

Fig. 3, a cross section through a second embodiment.

Fig. 4, a perspective view of a device according to a third embodiment
15 placed within a vessel; and

Fig. 5, a cross-section of view of the device according to Fig. 4.



Fig 1 shows a cell culture device comprising a frame 10 and a cell culture substrate 14. The frame 10 comprises an open peripheral frame 11, which forms a planar portion of the frame, and upstanding walls 13. Walls 13 and peripheral frame 11 are formed integrally by vacuum forming a sheet of 200 μ m thick polyethylene terephthalate film. The walls 13 have a lip 12 extending around their upper edges. The cell culture substrate film 14 is releasably secured to the lower surface of peripheral frame 11 by lamination. The cell culture substrate comprises a conformable sheet of ethylene-vinyl acetate film which has been treated on its upper surface by a corona discharge technique to modify its surface properties for optimal cell attachment. The central portion of this upper surface is accessible through the open part of peripheral frame 11. The frame and culture substrate which form the device may be placed within a petri-dish 15 as shown in Fig 2. The frame and culture substrate are preferably sterile and supplied in a bacteria-proof package.

To use the cell culture device, it is placed in a petri-dish or other container and then a cell culture medium containing e.g. human epithelial keratinocytes is added. The cells are cultured at 37°C for a number of days during which the cells multiply and attach to the substrate until the required cell density and degree of confluence of the cell layer is achieved. The medium is then removed and the device is removed from the container. The cell culture substrate may then be peeled from the frame and placed, cell side downwards, on a wound to be treated.

In Fig 3, the culture substrate is attached over its lower surface to a support layer 16 which is a 150 μ m thick piece of PET film. The support provides additional rigidity to the film 14 to help maintain it in a flat and wrinkle-free condition. When the cells have been grown and attached to the culture substrate, the support and substrate are removed from the frame together and placed on a patient, whereupon the support layer may be removed by peeling.

In Fig 4 and 5, the cell culture substrate material 14 is peelably attached on its lower surface to support layer 16 over that part of

support layer 16 defined by the peripheral frame 11. To aid peelability, substrate material 14 is provided with a tab portion 17 towards a corner of substrate material 14. The remaining part of support layer 16 is fixedly attached to frame 11.

5

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any
10 other integer or step or group of integers or steps.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

5
10
15
20



THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. A cell culture substrate device comprising a frame member formed from a material which has no or low toxicity to cells and a cell culture substrate which is not toxic to cells and having at least one surface to which cells may attach, wherein said frame member is adapted to releasably secure said culture substrate in a suitable configuration of the culture substrate for attachment of cells to said at least one surface and wherein the frame member further comprises upstanding walls extending around the said surface to which cells may attach, wherein the walls further comprise means for retaining the frame member within a container.
2. The device of claim 1 wherein the culture substrate comprises a polymeric material which has a surface suitable for attachment of cells.
3. The device of claim 1 or 2 wherein the substrate comprises a film.
4. The device according to claim 2 or 3 when dependent therefrom wherein the polymeric material is selected from polyesters, polypropylenes, vinyl acetate copolymers, polyvinylidene chlorides, polystyrenes, polybutadienes, polyethylenes, ionomers, and blends and copolymers containing any of said polymers.
5. The device according to any preceding claim wherein the frame member is adapted to secure the culture substrate in a substantially flat configuration.
6. The device according to claim 5 wherein the frame member comprises a planar portion which engages at least a part of the cell culture substrate to releasably secure the substrate in a flat configuration in a plane parallel to the planar portion.



7. The device according to claim 5 or 6 wherein the frame member comprises a peripheral frame so as to define an open frame member.
8. The device according to any preceding claim wherein the means for retaining comprise a lip or recess which engages an edge of the container.
9. The device according to any preceding claim wherein the culture substrate is releasably secured to the frame member by adhesive bonding.
10. The device according to any preceding claim wherein the cell culture substrate further comprises a support layer releasably attached to a surface opposed to the at least one surface.
11. The device according to claim 10 wherein the opposed surface covers a portion of the support layer.
12. The device according to claim 11 wherein the opposed surface covers a substantially central portion of the support layer thereby defining an outer uncovered peripheral portion of the support layer.
13. The device according to claim 12 wherein the outer uncovered peripheral portion is fixedly attached to the frame member.
14. The device according to any preceding claim wherein the at least one surface further comprises a tab portion.
15. A cell culture substrate device substantially as herein described with reference to the accompanying drawings.
16. The device according to any preceding claim together with a batch of mammalian cells.



17. The use of the device according to any preceding claim.

18. A cell culture system comprises a container and a cell culture substrate
5 device, the device comprising a frame member formed from a material which
has no or low toxicity cells and a cell culture substrate which is not toxic to cells
and having at least one surface to which cells may attach, said frame member
being adapted to releasably secure said cell culture substrate in a suitable
10 configuration of the culture substrate for the attachment of cells to said surface,
wherein said frame member is of a shape and size to fit into said container, and
said frame member has means for being retained within the container, such that
said cell culture substrate is held substantially parallel to one internal surface of
said container.

19. A cell culture system wherein the cell culture substrate device is in
15 accordance with any one of claims 1 to 16.

DATED this 14th day of February 2001

20 **SMITH & NEPHEW, PLC**

by DAVIES COLLISON CAVE
Patent Attorneys for the Applicants

SECRET



1/2

FIG. 1.

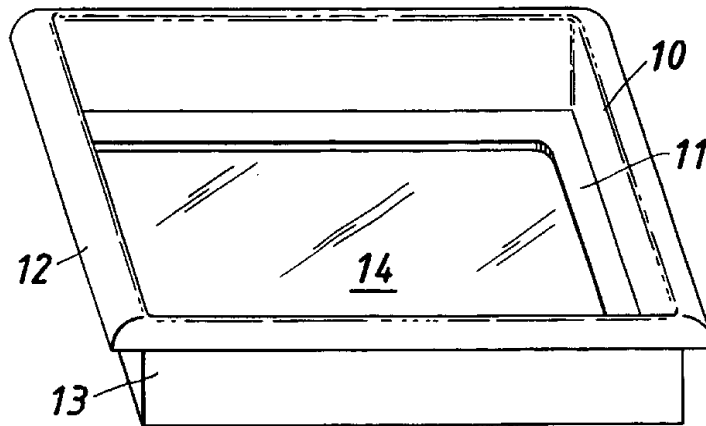


FIG. 2.

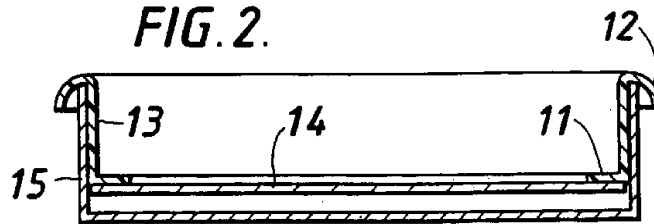


FIG. 3.

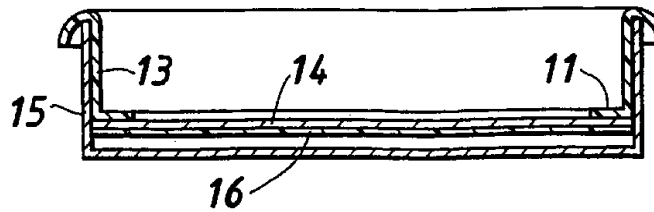


FIG. 4.

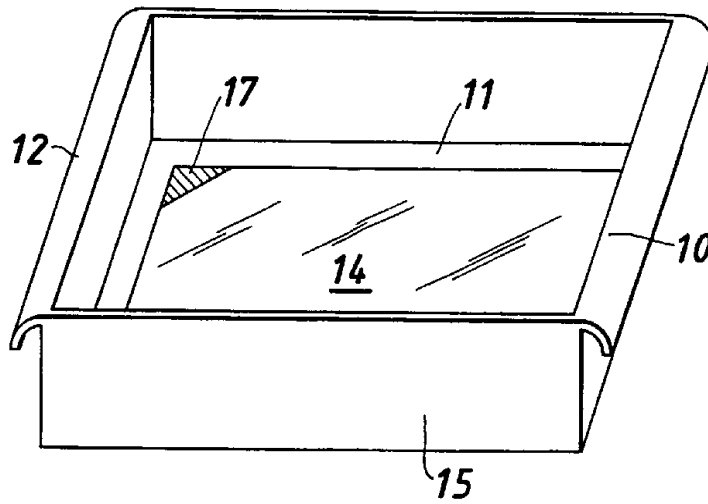


FIG. 5.

