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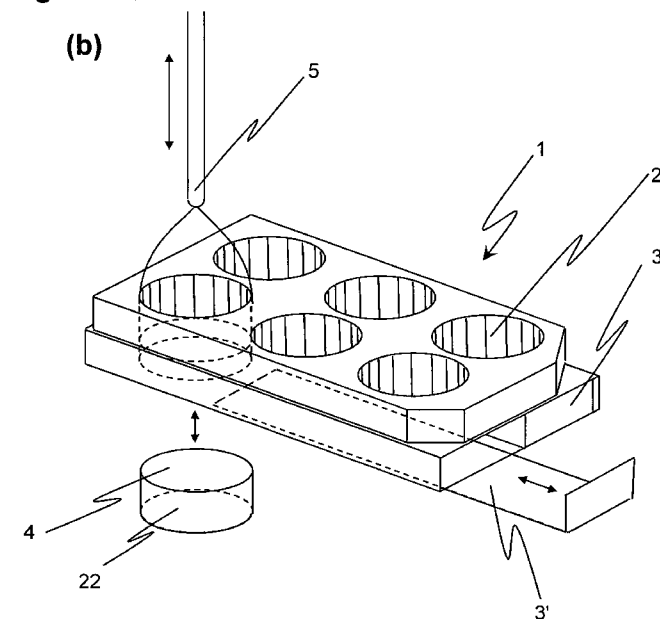
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(54) Title: MULTIWELL CULTURE PLATE FOR THREE-DIMENSIONAL CULTURES

Fig. 2



(57) Abstract: In a multiwell plate 1 the multitude of cavities 2 are each formed by a rigid wall 21 and a flat bottom part 22, said bottom part being hermetically bonded to the cavity wall 21, while at the same time individually detachable from the wall 21 along its rim 23. To transfer a three-dimensional culture 4 to a second device, the bottom part 22 of the cavity 2 is individually detached from the cavity wall with a suitable device 5, that allows to detach the bottom part along its rim, e.g. by applying pressure. The three-dimensional culture 4 remains on the detached bottom part, while the multiwell plate with the cavity wall is lifted away. Since the culture 4 itself is not mechanically strained, no damage to the culture can take place.

Multiwell Culture Plate for three-dimensional cultures

Field of the Invention

The invention relates to multiwell culture plates for growing cell or tissue cultures in vitro, and a method for transferring a cell or tissue culture from a multiwell plate, according to the preambles of the independent claims.

State of the art

To cultivate and/or investigate a number of cell cultures in parallel, multiwell culture plates are used, consisting of a plate body having a multitudes of wells. Industry standard multiwell plates are laid out with 96 wells, arranged in an 8 x 12 matrix. Other variants of multiwell plates are available, e.g. with 6, 12, 24, 48, 96, 384, or 1536 wells. The size of the wells, the distance between the rows and columns, as well as the height, length and width of said multiwell plates are standardized. A large number of auxiliary equipment is available for such standard size multiwell plates, including devices that load and unload precise volumes of liquid in multiples of 8, 12, or 96 wells at a time. Most multiwell plates for cell cultures are consumable goods intended for single-use, manufactured of suitable plastic material such as polystyrene.

A number of different types of multiwell culture plates intended for specific purposes have been developed. For example WO 86/07606 discloses a multiwell plate with wells that contain an opening in the bottom, and a filter membrane arranged at that opening. Such a multiwell plate is intended to gain the liquid medium of a number of bacteria or cell cultures in parallel. WO 98/35013 shows a multiwell plate with wells that are di-

vided into two chambers by a semi-permeable membrane placed in the middle of the well.

The known multiwell culture plates essentially comprise a rigid plate frame structure with a multitude of cavities, regularly arranged on the plate body, each cavity having an opening on top of the plate. A cover can be placed on the plate in order to protect the
5 cultures from contamination. The multiwell plates have to be completely sterile, which is achieved for example by treating the multiwell plates in their sealed packages with gamma radiation.

While the use of multiwell plates for cultivating cells in liquid media is very convenient
10 and highly automizable, this is so far not the case for three-dimensional culture types, as for example three-dimensional collagen stem cell cultures, or chick spinal cord tissue cultures in agarose. If such a non-liquid culture has to be transferred to a second device, for example to an embedding mould, this cannot be achieved by simply using a pipette, as it would be possible for cultures in fluid media. At the present there are only few
15 ways to transfer a three-dimensional culture, for example by simply turning the multiwell plate upside down and knocking on the plate, letting the sample fall out of the well. With this method all cell cultures of the plate have to be removed at the same time. Another way to remove the sample culture is to lift it with forceps. One may even scrap the culture from the well. All the current techniques lead to mechanical stress and physical
20 deformation of the three-dimensional cell culture. Cells, however, are very susceptible to shear forces, and thus may be damaged during transfer from the multi-well plate. As a consequence this may lead to irreproducible alterations of the experimental results.

In addition the known techniques are time consuming, and can only be carried out manually. In today's industrial laboratory practice, however, automation is a very important measure to increase throughput and to decrease costs.

Another problem is the lack of reproducibility of such manual transfer steps, particularly its influence on the cell culture. As a consequence the culturing of three-dimensional cell samples cannot be standardized to the same degree as it is possible for cultures in liquid media.

Objects of the Invention

An object of the invention is to provide multiwell culture plates for growing cell or tissue cultures in vitro that do not have the above-mentioned and other disadvantages, and particularly do allow the transfer of cell cultures from specific wells of the multiwell plate with reduced mechanical stress or physical deformation. Another object of the invention is to provide a method for transferring a cell or tissue culture from a multiwell plate, in which the cultures are subject to less mechanical stress or physical deformation.

In addition a multiwell culture plate according to the invention and the method according to the invention should give the possibility to automatize such transfers, for example by using laboratory robots. Another object of the invention is an improved reproducibility of the transfer step, particularly its influence on the cell culture.

These and other objects are achieved by a multiwell plate and a method according to the independent claims. Advantageous embodiments are given in the dependent claims.

Summary of the invention

In a multiwell plate according to the invention the cavities are each formed by a rigid wall and a preferably flat bottom part, said bottom part being hermetically bonded to the cavity wall, while at the same time being severable or sectionable from the wall along its rim. To transfer a three-dimensional culture to a second container, the bottom part of the cavity is detached from the cavity wall with a suitable detaching device that allows to sever, to section or to punch the bottom part along its rim, or to break the bonding between bottom and wall, e.g. by applying pressure, or to break a predetermined breaking point along the rim of the bottom part. The three-dimensional culture remains on the detached bottom part, while the multiwell plate is lifted away, perpendicular to the bottom part. An important characteristics of the invention is that the bottom part of each individual cavity consists of a thin, transparent, non-porous, cell culture compatible bottom that can be individually removed from the multiwell plate for example by a cutting device, together with the cell or tissue culture 4 that had been cultured on it.

Since the three-dimensional culture itself is not mechanically strained, no damage to the culture can take place. Subsequently the culture may be transferred to the second device together with the bottom part. Such multiwell plates according to the invention are especially advantageous for three-dimensional cell cultures, organotypic cultures, tissue engineering and regeneration, scaffold generation and synthesis, three-dimensional cultures of organs or parts of organs, e.g. chicken spinal cord in a agarose matrix, and imaging of three-dimensional biological structures.

Like the cavity wall, which can be made out of polystyrene, the bottom part has to be waterproof, non-porous, and cell culture compatible. Preferably the bottom part is a semi-flexible sheet or membrane. Even more preferably, the bottom part is a thin membrane, most preferably five to five hundred micrometers thin. Suitable materials for the bottom part are for example polycarbonate, polyester, or polytetrafluoroethylene. Espe-

cially preferred materials are polystyrene and polyethylene. The membrane must be compatible with routine tissue embedding and sectioning procedures, such as embedding in paraffin and sectioning with a cryomicrotome or a microtome for paraffin sections. The bottom part of the cavity may be attached to the cavity wall for example by
5 adhesive, or thermal or ultrasonic welding. When the bottom part is realized as a semi-flexible membrane, it is possible to attach one sheet of membrane, subsequently forming the bottom parts of several cavities. It is also possible to attach the membrane with a clamping mechanism as shown for example in WO 86/07606. In an advantageous embodiment of the invention the bottom part is translucent or transparent, to allow micro-
10 copy of the culture sample while illuminating the sample through the bottom part.

In another advantageous embodiment the multiwell plate comprises a drawer element arranged on the plate frame structure close to the bottom parts of the cavities. During normal handling, e.g. transporting or storing the multiwell plate, the drawer element remains in an inserted position, protecting the bottom part of the cavities from damages.
15 For transferring a culture from a specific cavity, the drawer element is retracted as far as necessary to free the specific cavity bottom. After removing the culture, the drawer is closed again, protecting the remaining cavity bottom parts. Instead of one drawer element, two or more drawer elements may be used, each giving e.g. access to one or more rows or columns of the multiwell plate according to the invention.

20 A method according to the invention for transferring a cell or tissue culture from a multiwell plate with a multitude of cavities, having their only opening oriented towards a first side of the multiwell plate, basically comprises the steps of (a) detaching the bottom part from the wall of a specific cavity, thereby forming a new opening in the cavity opposite to the first side of the plate; and (b) removing the culture through said new
25 opening together with the detached cavity bottom part. Preferably the bottom part is

detached by severing, sectioning, or punching the bottom part along its rim, or by breaking the bonding between the bottom part and the wall, or by breaking a predetermined breaking point along the rim of the bottom part.

Since the culture is not subject to mechanical stress and is not deformed, the negative
5 influence of the transfer step is much less than with the methods known from the prior art. The method can be carried out in a standardized, reproducible manner, thereby decreasing irreproducible errors in the experiment. Furthermore the method can be easily carried out by laboratory robots, and is highly automizable.

Brief description of the drawings

10 Exemplary embodiments of the invention will be described by means of figures:

Figure 1 schematically shows a preferred embodiment of a multiwell plate according to the invention, having two drawer elements, (a) with fully inserted drawer elements, and (b) with one drawer element partially pulled out, and one three-dimensional culture removed.

15 Figure 2 discloses (a) a device for severing or sectioning the bottom part of a well of a multiwell plate according to the invention along its rim, and (b) the application of said device

Figure 3 shows yet another embodiment of a multiwell plate according to the invention, with a reusable plate structure and modular, replaceable cavity inserts,
20 (a) in a side-view, and (b) in a transverse sectional view.

Figure 4 shows a transverse sectional view of yet another embodiment, with modular cavity inserts and drawers.

Figure 5 shows a possible application of a multiwell plate according to the invention.

Description of embodiments of the invention

5 An embodiment of a multiwell plate 1 according to the invention with two drawer elements 3, 3', is schematically shown in Figure 1(a) with fully inserted drawer elements 3, 3', and in Figure 1(b) with one drawer element 3' partially pulled out, and one three-dimensional culture 4 removed from the cavity 2'. The shown multiwell plate 1 comprises a plate frame structure 11 and six cavities 2. The cavities 2 comprise a cylindrical
10 wall 21 and a bottom part 22, said bottom part being severable or sectionable along its rim 23. In the shown embodiment the bottom part is a semi-flexible membrane, which is stable enough to carry the weight of heavy tissue scaffolds without being damaged, and thin enough to be easily detached from the cavity wall when necessary. The multiwell plate 1 also comprises a cover 12, shown above the plate 1. Said cover 12 has no effect
15 on the function of the invention itself. Two drawer elements 3, 3' are arranged in the plate frame structure 11, below the bottom parts 22. The drawer elements 3, 3' comprise a handle 31 and a drawer base 32, and are movable on tracks along the long axis of the multiwell plate 1. For a compact design the drawer base 32 is advantageously positioned close to the bottom part membrane 22, but preferably has no direct contact with
20 the membrane, to avoid unnecessary mechanical movements of the culture when the drawer is opened and closed.

To remove a culture 4 from a certain cavity 2' a special device 5 as shown in Figure 2 may be used. Such a device 8 essentially consists of a handle 52 and a circularly ar-

ranged blade or wire 51. In a first step the multiwell plate 1 is placed on a suitable flat surface, and the drawer element 3' is partially pulled out. In a second step the device 5 is used to sever or cut the bottom part membrane 22. The blade or wire 52 of the device 5 has a diameter that is slightly smaller than the inner diameter of the cylindrical cavity wall 21. In a third step, after detaching the bottom part 22 from the cavity 2, the multi-
5 well plate 1 is lifted, and the three-dimensional culture 4 remains on the surface, together with the detached bottom part membrane 22.

Another embodiment of a multiwell plate 1 according to the invention is schematically shown in Figure 3(a) in a side-view, and Figure 3(b) in a transverse sectional view. In
10 this embodiment of the invention the plate frame structure 11 may be reused, while only the replaceable cavities 2 are single-use. The multiwell plate 1 comprises a plate frame structure 11 with six circular support structures 13 for cavity inserts 24. Said single-use cavity inserts form the cavities 2" of the multiwell plate, comprising a cylindrical wall 21 and bottom part 22. The bottom part 22 is again severable or sectionable from the
15 cavity 2 along its rim 23. The cavity insert 24 is placed on the support structure 13. The shown embodiment comprises no drawers, but is permanently closed by a plate base 15. To transfer a culture sample to a second device, the insert 24 with cavity 2" holding the culture is removed from the multiwell plate 1 and placed on surface. The bottom part 22 is then detached from the cavity 2" in the same manner as for the embodiment shown in
20 Figure 1.

In yet another embodiment, shown in Figure 4 in a cross-section, the cavities are modular as in the embodiment of Figure 3, and the plate structure 1 comprises drawers 3, 3', as in the embodiment of Figure 1. Another advantage of modular cavity inserts is that each cavity may be equipped with its own cover.

The application of a multiwell plate 1 according to the invention is described in Figure 5. In a first step the cover 12 is removed from the plate 1, and cell samples 6 are placed in the cavities 2, together with a gel or a scaffold as the three-dimensional medium. In a second step the cover 12 is closed, and the cells are cultured. In a third step the cover 12
5 is removed and the drawer 3 is partially retracted. The culture sample 4 is then removed, together with the bottom part 22, and further processed (fourth step), e.g. placed in an embedding mould. In a fifth step the cover 12 is closed again and the culturing of the remaining cell cultures is continued.

List of Reference Numerals

10	1	multiwell plate
	11	plate frame structure
	12	plate cover
	13	cavity support structure
	15	plate base
15	2, 2', 2"	cavity
	21	cavity wall
	22	bottom part
	23	rim
	24	cavity insert
20	3, 3'	drawer element
	31	handle
	32	drawer base
	4	three-dimensional culture
	5	detaching device
25	51	circular blade or wire

52 handle

Claims

1. A multiwell plate 1 for growing cell or tissue cultures 4 in vitro, with a plate frame structure 11, and a multitude of cavities 2, 2', 2'', their only opening oriented towards one side of the multiwell plate 1, wherein each cavity 2, 2', 2'' is formed by a cylindrical wall 21, and a bottom part 22, hermetically attached to the wall 21, **characterised in that** the bottom part 22 is individually detachable from the wall 21 of each cavity 2, 2', 2''.
2. The multiwell plate according to claim 1, **characterised in that** the bottom part 22 is a translucent or transparent membrane.
3. The multiwell plate according to claim 1 or 2, **characterised in that** the bottom part 22 is a semi-flexible, non-permeable membrane.
4. The multiwell plate according to any one of the preceding claims, **characterised in that** the bottom part 22 is bonded to the wall 21 by adhesive, thermal welding, or ultrasonic welding.
5. The multiwell plate according to any one of the preceding claims, **characterised in that** the bottom part 22 is detachable from the wall 21 by severing or sectioning the bottom part 22 along its rim 23, or by breaking the bonding between the bottom part 22 and the wall 21.
6. The multiwell plate according to any one of the preceding claims, **characterised by** one or more drawer elements 3, 3' that are moveably arranged in the plate frame structure 11 on the side opposite to the openings of the cavities 2, protecting the bottom parts 22 of the cavities 2.

7. The multiwell plate according to any one of the preceding claims, **characterised in that** the cavities 2 are realised as cavity inserts 24, which are removably seated on support structures 13 arranged on the plate frame structure 11.
8. The multiwell plate according to any one of the preceding claims, **characterised in that** the bottom part 22 is made from polystyrene, polyethylene, polycarbonate, polyester, or polytetrafluoroethylene.
9. A method for transferring a cell or tissue culture 4 from a multiwell plate 1, the multiwell plate 1 comprising a plate frame structure 11, and a multitude of cavities 2, 2', 2'' formed by a cylindrical wall 21 and a bottom part 22, hermetically attached to the wall 21, wherein said multitude of cavities have their only opening oriented towards a first side of the multiwell plate 1, **characterized in that**
- a. the bottom part 22 is individually detached from the wall 21 of a specific cavity 2, 2', 2'' thereby forming a new opening in the cavity 2, 2', 2'' opposite to the first side of the plate 1; and
 - b. the culture 4 is removed through said new opening together with the detached cavity bottom part 22.
10. The method according to claim 8, **characterized in that** the bottom part 22 is detached by severing, sectioning, or punching the bottom part 22 along its rim 23, or by breaking the bonding between the bottom part 22 and the wall 21, or by breaking a predetermined breaking point along the rim 23 of the bottom part 22.
11. The method according to claim 8 or 9, **characterized in that** the multiwell plate 1 is a multiwell plate according to any one of the claims 1 to 7.

Fig. 1 (a)

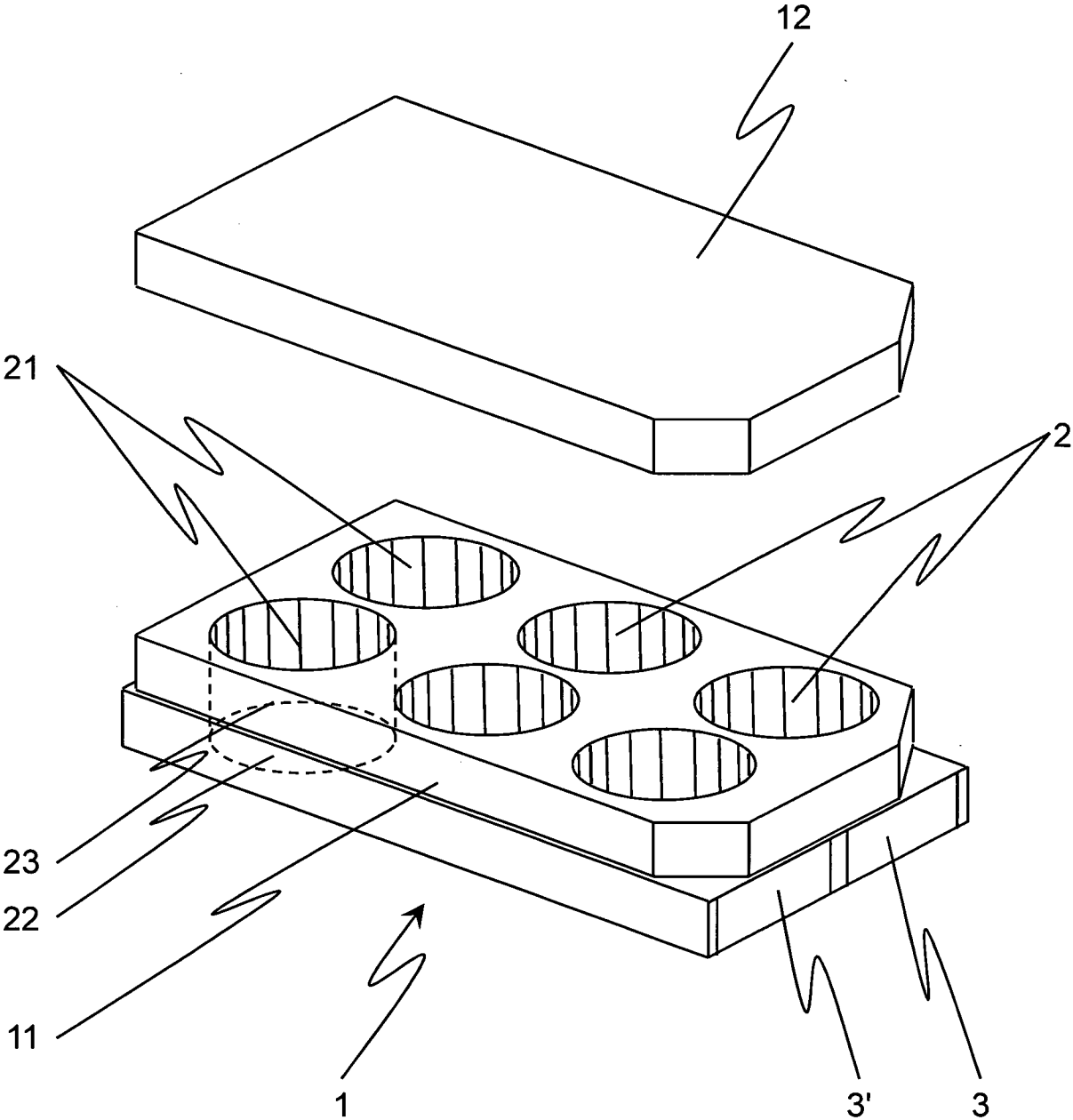


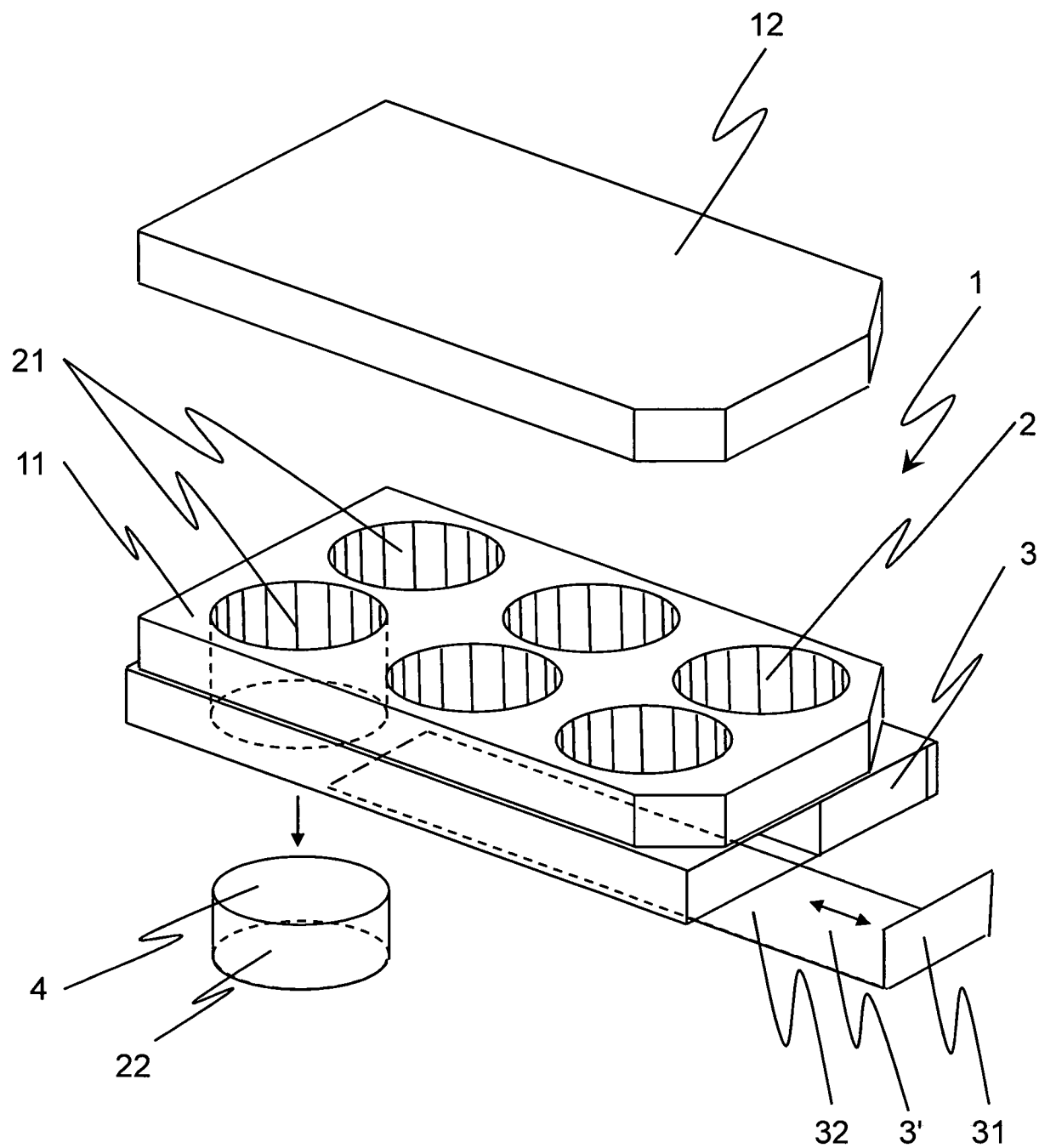
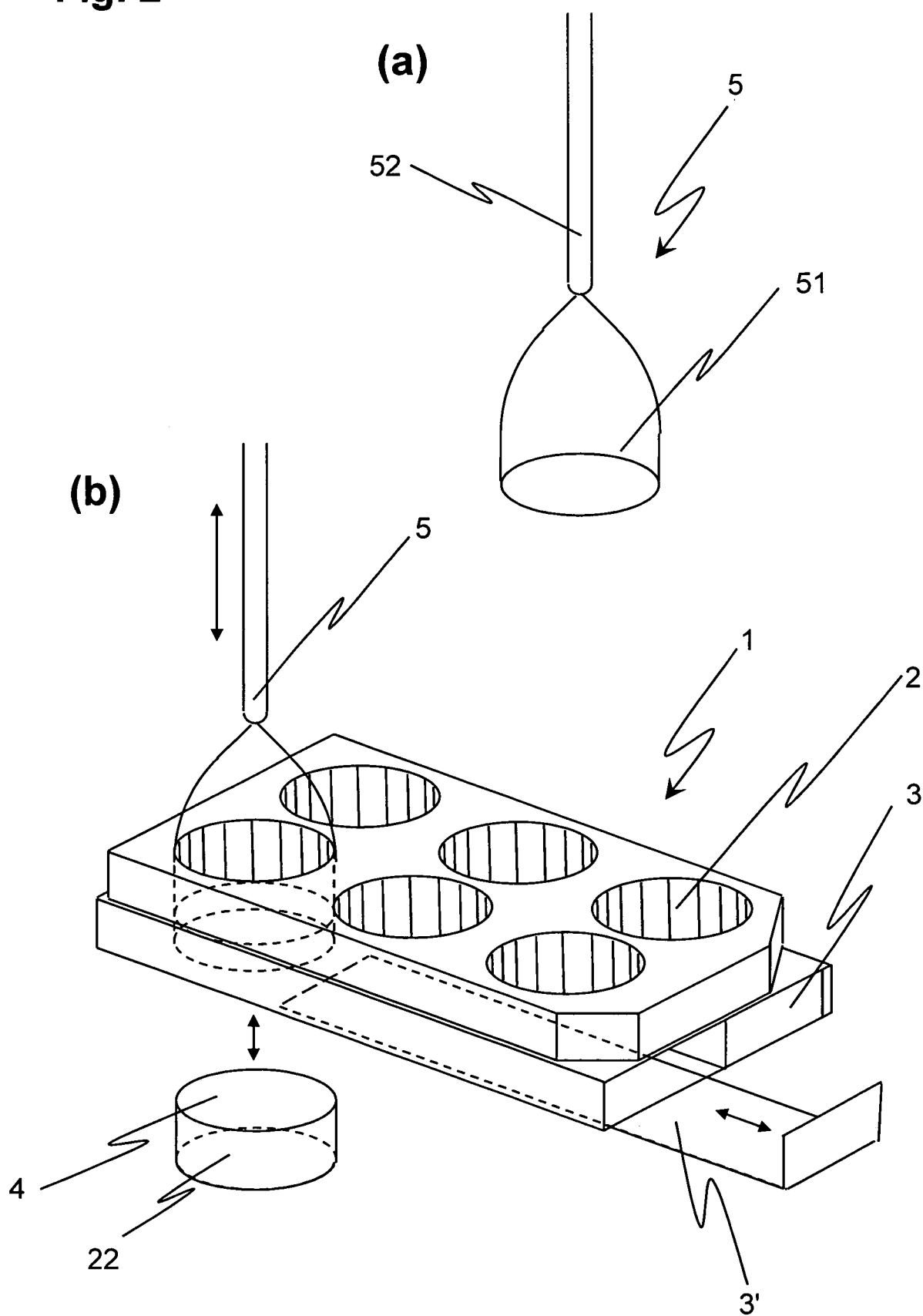
Fig. 1 (b)

Fig. 2

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Fig. 4

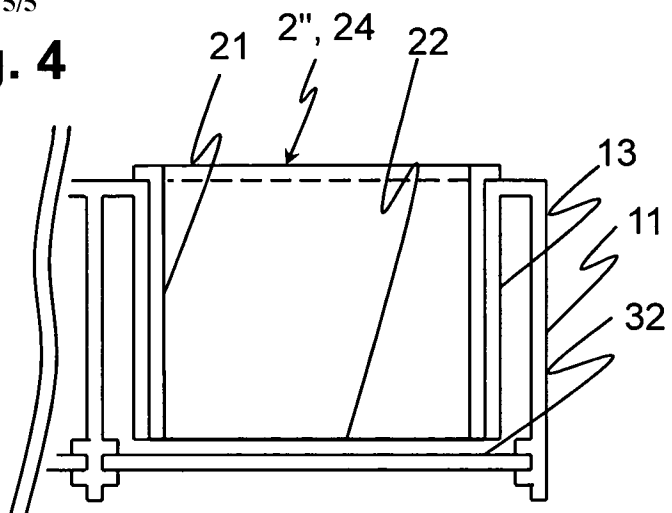
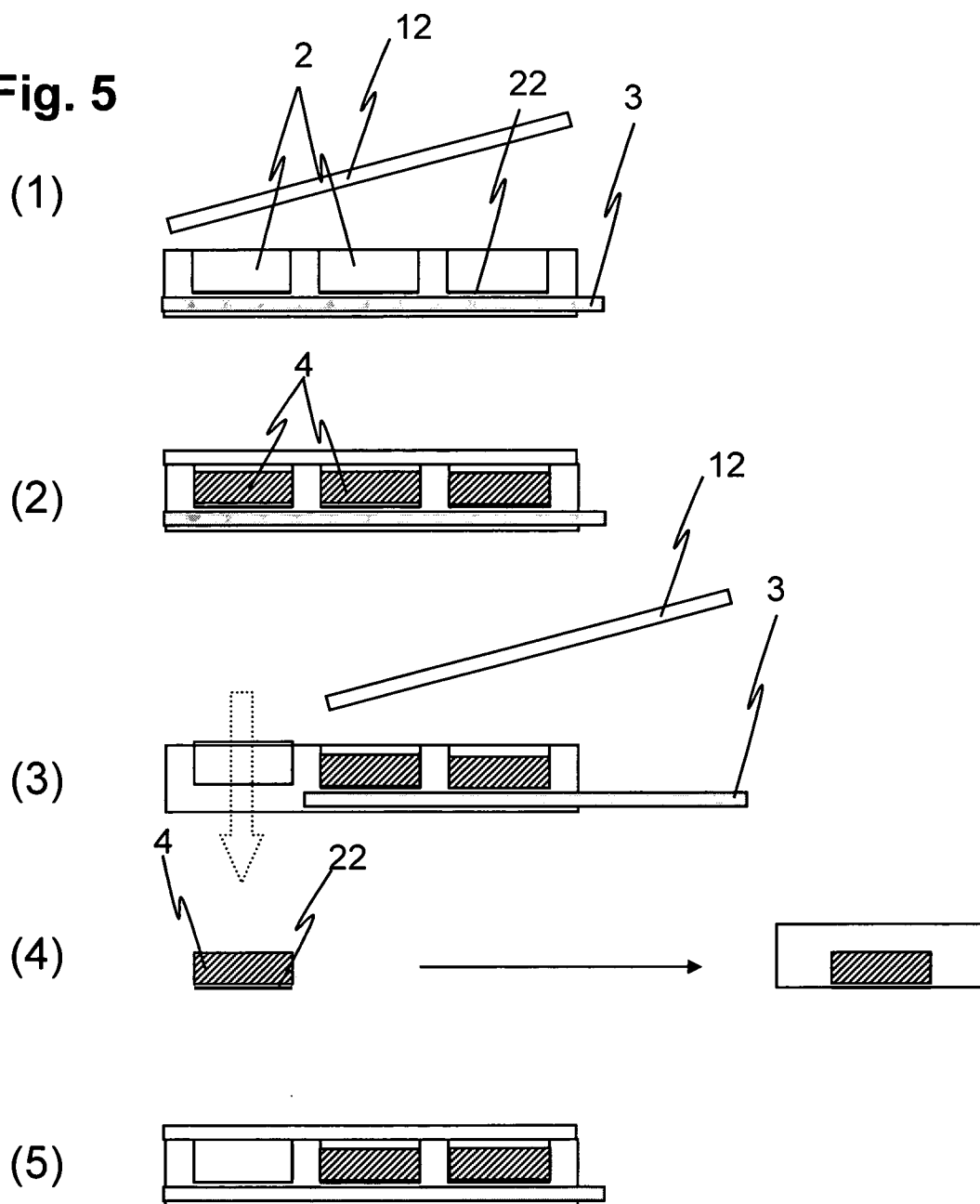


Fig. 5



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/000724

A. CLASSIFICATION OF SUBJECT MATTER
INV. B01L3/00 C12M1/16

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)
B01L C12M

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)

EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 1 232 792 A (HOFFMANN LA ROCHE [CH]; WEIDMANN PLASTICS TECHNOLOGY A [CH]) 21 August 2002 (2002-08-21) paragraphs [0013] - [0028], [0039]; claim 1; figures 1,2 the whole document	1-8
X	EP 1 358 937 A (MILLIPORE CORP [US]) 5 November 2003 (2003-11-05) paragraphs [0003], [0009], [0010], [0013]; figure 5	1-6,8
A	US 4 299 920 A (PETERS J HINRICH) 10 November 1981 (1981-11-10) column 1, lines 5-45 column 2, lines 28-36	1-11
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☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2009/000724

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2003/032046 A1 (DUFFY DAVID [US] ET AL DUFFY DAVID [US] ET AL) 13 February 2003 (2003-02-13) paragraphs [0015], [0016] -----	1-11
A	EP 1 477 792 A (BECTON DICKINSON CO [US]) 17 November 2004 (2004-11-17) paragraph [0019] -----	
A	US 3 883 398 A (ONO KAZUYUKI RAY) 13 May 1975 (1975-05-13) the whole document figure 1 -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/EP2009/000724

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
EP 1232792	A	21-08-2002	DE 60122854 T2	08-03-2007
			JP 2002323502 A	08-11-2002
			US 2002155035 A1	24-10-2002
			US 2005249640 A1	10-11-2005
EP 1358937	A	05-11-2003	DE 60317603 T2	23-10-2008
			ES 2294244 T3	01-04-2008
			JP 3899049 B2	28-03-2007
			JP 2004004066 A	08-01-2004
			US 2003205511 A1	06-11-2003
US 4299920	A	10-11-1981	AU 5471080 A	24-07-1980
			CA 1167419 A1	15-05-1984
			DE 2902026 A1	24-07-1980
			DK 16280 A	20-07-1980
			EP 0014007 A1	06-08-1980
			ES 8201203 A1	01-03-1982
			FI 800097 A	20-07-1980
			IL 59075 A	30-09-1982
			JP 55108284 A	20-08-1980
			NO 800089 A	21-07-1980
US 2003032046	A1	13-02-2003	US 2005250097 A1	10-11-2005
			US 2003073228 A1	17-04-2003
			US 2005100974 A1	12-05-2005
			US 2003032076 A1	13-02-2003
			US 2003068637 A1	10-04-2003
EP 1477792	A	17-11-2004	AU 2004202003 A1	02-12-2004
			CA 2467164 A1	13-11-2004
			DE 602004002888 T2	31-05-2007
			ES 2270279 T3	01-04-2007
			JP 2004340971 A	02-12-2004
US 3883398	A	13-05-1975	NONE	