(12) STANDARD PATENT (11) Application No. **AU 2003270995 B2**
(19) AUSTRALIAN PATENT OFFICE

(54) Title
A thermoformed container for the culturing of cells

(51) International Patent Classification(s)
C12M 1/00 (2006.01) ***C12M 1/22*** (2006.01)
B29C 51/14 (2006.01) ***C12M 3/00*** (2006.01)
B32B 37/00 (2006.01) *B01L 3/00* (2006.01)

(21) Application No: **2003270995** (22) Date of Filing: **2003.12.18**

(30) Priority Data

(31) Number	(32) Date	(33) Country
02 16439	**2002.12.20**	**FR**

(43) Publication Date: **2004.07.08**
(43) Publication Journal Date: **2004.07.08**
(44) Accepted Journal Date: **2011.09.22**

(71) Applicant(s)
Maco Pharma

(72) Inventor(s)
Heron, Antoine

(74) Agent / Attorney
Allens Arthur Robinson Patent & Trade Marks Attorneys, Level 27 530 Collins Street, Melbourne, VIC, 3000

(56) Related Art
US 5512480
US 6297046
US 6245555
EP 0890636

ABSTRACT

The invention relates to a container (1) intended for the culturing of cells, comprising a first (2) and a second (3) sheet that are secured to one another in the vicinity of their periphery so as to form an interior volume intended to receive the cells, and at least one access route designed to allow the introduction and/or the recovery of the cells.

Each of said sheets comprises at least one layer made of a polymer material that allows the cells to adhere. Moreover, at least one of the two sheets is thermoformed.

The invention also relates to a system comprising at least two elements connected together as a closed circuit by means of a tube, one of said elements being such a container, and also to the use of such a container or of such a system for culturing adhering cells and/or cells that are in suspension in the medium.

Figure 1

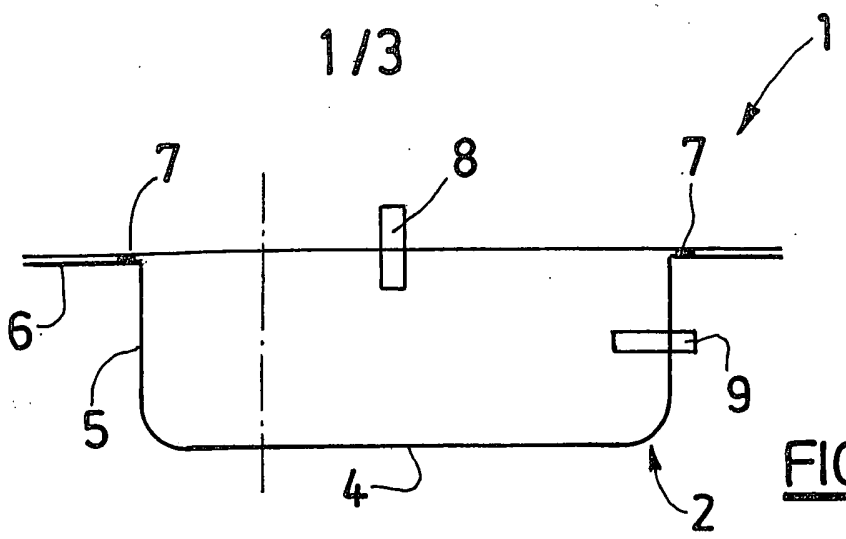

FIG.1

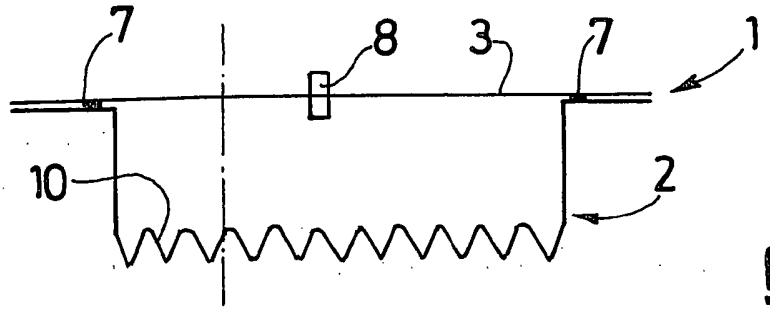

FIG.2a

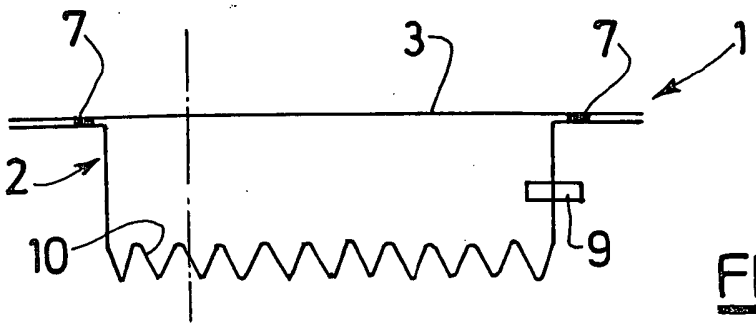

FIG.2b

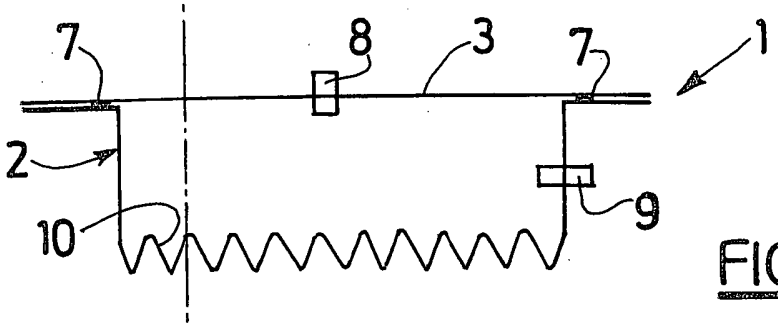

FIG.2c

P/00/011

Regulation 3.2

AUSTRALIA

Patents Act 1990

ORIGINAL

COMPLETE SPECIFICATION

STANDARD PATENT

Invention title: A thermoformed container for the culturing of cells

The following statement is a full description of this invention, including the best method of performing it known to us:

A thermoformed container for the culturing of cells

Field of the Invention

The invention relates to a container intended for the culturing of cells, to a system comprising at least two elements connected together as a closed circuit by means of a tube, one of said elements being such a container, and also to the use of such a container or of such a system for culturing cells.

The container according to the invention is more particularly intended for the culturing of cells by adhesion to the inner surface of the container. Of course, the container may also be used for culturing cells in suspension in a medium contained in the container.

Background of the Invention

In this specification, where a document, act or item of knowledge is referred to or discussed, this reference or discussion is not an admission that the document, act or item of knowledge or any combination thereof was at the priority date:

(i) part of common general knowledge; or

(ii) known to be relevant to an attempt to solve any problem with which this specification is concerned.

The advent of in vitro cultures of cells that can be directly transplanted into humans is at the origin of the development of different types of container for packaging said cultures. Used in a medical context, to prepare products for use in cell and gene therapy, these containers must have guarantees in terms of confining the cells and preventing any risks of contamination and technical handling errors. Said containers must therefore comply with strict good practice regulations for transfusable products in respect of closed packaging and cell transfer.

The use of flexible pouches has grown for culturing cells used in human therapy, particularly in the context of developing clinical protocols for the ex vivo expansion of haematopoietic cells derived from a sample of bone marrow, peripheral blood or umbilical cord blood.

Flexible pouches comply with the abovementioned good practice regulations but have disadvantages in respect of culturing cells.

Firstly, the flexibility of these pouches does not allow them to be conveniently stacked within an incubator.

Secondly, the flexibility of a pouch defines a culture surface that is flexible and therefore deformable as a function of the level of filling and the handling operations. This deformability causes zones of sedimentation and a heterogeneous distribution of the cells over the available culture surface.

Furthermore, the available culture surface is limited by the size of the pouch. In order to satisfy certain applications that require large culture surfaces, the increase in the size of a pouch or the multiplicity of small pouches considerably increases the difficulties encountered during handling.

Finally, the gas-permeable materials conventionally used for flexible pouches for cell culturing, of the polyethylene, polypropylene, fluoropolymer and ethylene-vinyl acetate (EVA) type, do not allow the culturing of adhering cells but only the culturing of cells in suspension in the medium, and this considerably limits the applications that are possible. This is because most cells of interest are cells which are cultured by adhesion.

Moreover, the document US 6 297 046 discloses a flexible pouch intended for the culturing of cells, in particular by adhesion. Essentially, the pouch is formed by the association of two sheets that are themselves made of a complex of two films, one of which defines an adhesive inner surface for the cells. The lower flexibility of adhesive polymer films necessitates the use of very thin films to produce a flexible pouch, hence the need to complex them. Furthermore, the use of complex sheets limits the transparency of the pouch and therefore the possibility of observing the cell development under a microscope. Besides the difficulties in producing the pouch described in said document, this pouch only aims to solve the problem of adhesion of the cells, whereas the user would like a global response to all of the problems mentioned above.

Summary of the invention

According to a first aspect of the present invention there is provided a container intended for the culturing of cells, comprising a first and a second gas-permeable sheet that are secured to one another in the vicinity of their periphery so as to form an interior volume intended to receive the cells, and at least one access route designed to allow the introduction and/or the recovery of the cells.

Preferred embodiments of the invention provide a container that complies with the good practice regulations mentioned above while allowing the culturing of cells by adhesion,a container that is stackable, and provides increased and homogeneous culture surfaces in a container of small size.

According to the invention, each of said sheets comprises at least one layer made of a polymer material that allows the cells to adhere, and at least one of the two sheets is thermoformed.

The fact of producing a container from at least one sheet of adhesive polymer material using the technique of thermoforming gives the container particularly beneficial characteristics, including:

- the container has a defined geometry, which allows a number of containers to be conveniently stacked within an incubator;
- the container has a certain rigidity, which makes it possible to avoid creating zones of preferential sedimentation and a heterogeneity of distribution of the cells over the available culture surface.

In one particular embodiment, each sheet is formed essentially of a polymer material that allows the cells to adhere. The technique of thermoforming means that it is not necessary to use complex sheets.

According to one variant of the invention, at least one of the thermoformed sheets has reliefs that are arranged in the interior volume of the container.

The technique of thermoforming makes it possible to produce reliefs arranged in the interior volume of the container. Thus, it is possible to considerably increase the available culture surface without increasing the size of the container and without increasing the volume of culture medium consumed.

These reliefs may form repeating or irregular, continuous or separate motifs.

According to one possible embodiment, the sheets are secured to one another by welding in the vicinity of their periphery.

For example, at least a first sheet is thermoformed so as to have, in transverse section, the overall shape of a rectangle with rounded corners comprising a substantially flat base, a side wall and a peripheral wall that forms a rim.

The second sheet, which forms the upper wall of the container, may be:

- either secured to the rim of the first sheet so as to be substantially flat;

- or thermoformed so as to have a geometry which is the same as that of the first sheet, said sheets being secured facing one another by their rims.

The container may comprise at least one access route that communicates with the interior volume of the container through a wall of a thermoformed sheet, for example a side wall, peripheral wall or upper wall.

According to a second aspect, the invention relates to a system comprising at least two elements connected together as a closed circuit by means of a tube, at least one of said elements being a container as described above, the tube being connected at a first end to the access route of the container and at a second end to another element of the system, so as to allow the cells and/or fluids to pass between the elements of the system.

Finally, according to a third aspect, the invention relates to the use of such a container or of such a system for culturing adhering cells or cells that are in suspension in the medium.

Description of the Preferred Embodiment

The other characteristics of the invention emerge from the following description of some embodiments, given with reference to the appended figures, in which:

- figure 1 is a schematic representation in transverse section of a container formed of a thermoformed sheet and of a sheet, the container being provided with two access routes;

- figures 2a to 2c are schematic representations in transverse section of containers formed of a thermoformed sheet having reliefs on its inner face and of a sheet, the containers being provided with one or two access routes;

- figures 3a to 3f are schematic representations in transverse section of containers formed of two thermoformed sheets, which do or do not have reliefs, the containers being provided with one or two access routes;

- figure 4a is a schematic representation in transverse section of a container formed of a thermoformed sheet and of a sheet, the container being provided, on the peripheral wall of the thermoformed sheet, with one access route that is oriented vertically downwards;

- figure 4b is a schematic representation in transverse section of a container formed of two thermoformed sheets, the container being provided, on the peripheral wall of

one of the thermoformed sheets, with one access route that is oriented vertically upwards;

- figure 4c schematically shows the structure of an access route;
- figure 4d schematically shows the structure of an access route associated with an inner reinforcement;
- figures 5a to 5d show various structures that the reliefs of the thermoformed sheet or sheets may have.

A container 1 according to the invention comprises two sheets 2, 3, lower and upper respectively, which are secured to one another in the vicinity of their periphery.

According to one possible embodiment, the sheets 2, 3 are welded. However, the sheets 2, 3 may also be secured by a different method, in particular by adhesive bonding.

The container 1 thus defines an interior volume that is intended to receive cells and a culture medium.

The two sheets 2, 3 are permeable to gases, in particular to oxygen, and are made of a transparent, biocompatible polymer material to which the cells can adhere. As such, the container 1 allows very good development of the cells, and its transparency offers the possibility of monitoring cell production using optical microscopy.

By way of example of a polymer material that can be used for the sheets 2, 3, mention may be made of polyester, in particular in APET or PETG form, polycarbonate or polystyrene.

Moreover, the potential for cell adhesion of the polymers used for the sheets 2, 3 may easily be increased by various known surface treatments, in particular chemical grafting, or a treatment using activating gases. Preferably, a surface treatment of the plasma type (plasma/oxygen or plasma/air) is carried out specifically on the surface intended for the culturing, before the container 1 is closed.

According to the invention, at least the lower sheet 2 is thermoformed. The container 1 has, in transverse section, the overall shape of a rectangle with rounded corners, comprising a wall that forms the bottom 4 of the container 1, said wall being surrounded by a side wall 5 which is extended laterally by a peripheral wall 6 that forms a rim intended to be secured to the upper sheet 3. The presence of these rounded corners guarantees optimal recovery of the cell products after culturing.

The sheets 2, 3 may have variable thicknesses and variable levels of gas permeability. For example, the thickness of one sheet is between 100 and 500 μm. This small thickness makes it possible to obtain a satisfactory permeability to gases, in particular to oxygen.

The thermoforming leads to a reduction in the thickness of the sheet. It is then possible to vary this phenomenon so as to vary the gas permeability of the container 1.

Moreover, the production of containers with various depths also makes it possible to vary the oxygenation of the medium. This is because certain cells, in particular haematopoietic cells, grow preferably in a medium with little oxygen: in this case, it is judicious to use a container of small depth that is filled exclusively with culture medium, so as to limit the exchange of gases. On the other hand, other types of cells, such as hepatocytes, consume a large amount of oxygen: a deeper container that is partially filled with culture medium then makes it possible to obtain an interface with a volume of air contained in the container, and consequently to promote the exchange of gases and in particular the supply of oxygen. In this context, the use of the thermoforming technique makes it possible to produce containers of various depths very easily, without it being necessary to create a mould for each type of container that is to be produced. A modification of the calibration of the mould is all that is required to modify the interior volume of the container.

According to a second aspect, the invention relates to a system comprising several elements, at least one of which is a container according to the invention, which elements are associated with one another so as to form a closed circuit. Such a system may in particular comprise elements that can sample, transfer, feed, concentrate, filter, inactivate or wash cell products. For example, these elements may be composed of flexible pouches for packaging media and reagents for cell culturing and of flexible pouches for transferring and centrifuging the cell products. In this context, the concept of a closed system that incorporates at least one container according to the invention is intended to make safer all the handling operations that are carried out to produce cells by culturing.

It may be judicious to limit the gas permeability of the container 1 when the latter is integrated in a system capable of piloting the supply of gases to cells. This permeability may be easily limited, in particular by using thicker sheets.

A piloting of the fluids within the system defines a cell culture bioreactor. In this context, the system is able to continuously or sequentially supply the cells being cultured by circulating the media and reagents. The system may also be equipped with a set of regulation and control means. These means make it possible in particular to apply the

values of time, temperature, pH of the medium and gas content that are selected for a given application. A pilotable system or bioreactor is most particularly beneficial for carrying out long-term cell culturing; mention may be made for example of the production of mesenchymal cells extracted from bone marrow or of the production of haematopoietic cells in coculture on adhering stromal cells.

According to a first embodiment of the invention, shown in figures 1 and 2a to 2c, the upper sheet 3 is not thermoformed.

The sheets 2, 3 are secured to one another at a welding zone 7 located for example on the peripheral wall 6 of the lower sheet 2, near the side wall 5. The upper sheet 3 is arranged so as to be substantially flat.

In figure 1, the lower sheet 2 does not have reliefs. The bottom 4, in particular, offers a homogeneous surface for the distribution and culturing of the cells, since it is substantially flat and smooth.

Moreover, the container 1 comprises two orifices designed to allow the introduction and/or the recovery of the cells, by means of access routes cooperating with said orifices. A first access route 8 communicates with the interior of the container 1 through the upper wall of the container 1, said upper wall being formed by the upper sheet 3, and a second access route 9 communicates with the interior of the container 1 through the side wall 5 of the thermoformed lower sheet 2.

In figures 2a to 2c, the lower sheet 2 has reliefs 10 on its face that lies within the container 1, essentially on the bottom 4, the bottom retaining a flat and homogeneous overall shape.

The reliefs 10 make it possible to solve the problem of the large surfaces needed to culture certain cells in a closed system. This is because the production of adhering human cells (mesenchymal, muscular, neural cells, etc.) is limited by the maximum density of the cells per unit surface beyond which cell proliferation ceases. The minimum number of cells required for a graft therefore necessitates a minimum cell culture surface, the latter usually having to be greater than one square metre.

The use of the thermoforming technique makes it possible to structure the cell culture surface and to considerably increase the available culture surface for a container 1 having the same size.

This makes it possible to understand the benefit of forming reliefs 10 on the bottom 4 and within the container 1, whereas the production of such reliefs 10 on the side walls 5 is not indispensable since the cells will settle on the bottom 4 by gravity.

In one particular embodiment, the sheets 2, 3, which have been thermoformed and structured with reliefs, are provided with flat zones in order to facilitate the observation of the cells under a microscope, and also where necessary the insertion of access routes.

The reliefs 10 may take various forms, as will be described below with reference to figures 5a to 5d.

The container 1 shown in figures 3a to 3f is formed of a thermoformed lower sheet 2 and of a likewise thermoformed upper sheet 3, said upper sheet 3 for example having a geometry that is the same as or substantially identical to that of the lower sheet 2. The sheets 2, 3 are secured facing one another.

The lower sheet 2 has reliefs 10 on its face within the container 1, it being possible for the upper sheet 3 also to have such reliefs 11 on its face within the container 1 (figures 3d, 3e, 3f) or, by contrast, to have a surface that is substantially flat and smooth (figures 3a, 3b, 3c). When the two sheets 2, 3 have reliefs 10, 11, the container 1 may be set down either on the lower sheet 2 or on the upper sheet 3 in order to culture cells by adhesion; the culturing of adhering cells may then be envisaged on contact with the two sheets 2, 3 at the same time, which doubles the already optimized capacity for cell production of said container 1.

Figure 4a shows a container 1 formed of a thermoformed lower sheet 2 that does not have reliefs and of an upper sheet 3, said sheets being secured to one another at a welding zone 7. The upper sheet 3 is arranged so as to be substantially flat.

Figure 4b is similar to figure 4a, although the upper sheet 3 is thermoformed and does not have reliefs.

Various embodiments can be conceived as regards the access routes. The container 1 may thus comprise either one access route 8 that communicates with the interior of the container 1 through the upper wall of the container 1 that is formed by the upper sheet 3 (figures 2a, 3a, 3d) or one access route 9 that communicates with the interior of the container 1 through the side wall 5 of the lower sheet 2 (figures 2b, 3b, 3e) or both access routes 8, 9 (figures 1, 2c, 3c, 3f).

The container 1 also has an access route 12 that is associated with the peripheral wall 6 of the lower sheet 2 (figure 4a) or with the peripheral wall of the upper sheet 3 (figure 4b).

The access routes 8, 9 are welded to the sheets 2, 3 but may also be secured to said sheets 2, 3 in particular by adhesive bonding.

However, these peripheral access routes 8, 9, 12 may be produced simply by the technique of thermoforming. Firstly, a protuberance is created on the wall of the sheet 2, 3. Then, the protuberances are perforated so as to create the access routes 8, 9, 12 and in particular allow a tube to be connected.

According to one possible embodiment, shown in figures 1, 2b, 2c, 3b, 3c, 3e and 3f, the protuberance is created on the side wall defined by the thermoforming of the lower sheet 2. The use of thermoforming moulds provided with removable parts makes it possible to extract the part thus formed.

According to another possible embodiment, shown in figures 4a and 4b, the protuberance is created on the sheet so as to be oriented perpendicular to the peripheral zone of the sheet, this orientation making it possible to facilitate production since the use of moulds provided with removable parts is then no longer necessary.

The production of the container according to the invention and of its access routes 8, 9, 12 makes it possible to circumvent the insertion of said access routes between the two sheets forming said container, as is the case in the manufacture of a flexible pouch. The zone where the two sheets are secured therefore remains entirely flat, and this constitutes an important element for welding materials that are adhesive for the cells, in particular non-complexed sheets based on polyester, polycarbonate or polystyrene films.

Furthermore, the integration of the access routes 8, 9, 12 in at least one thermoformed sheet makes it possible to eliminate the risks of leakage which may exist at the zone where said routes are inserted between the sheets of the containers of the prior art.

The production of the container 1 does not exclude the possibility of inserting the access routes, particularly in the form of tubes, between the two sheets 2 and 3 (not shown). Taking the preferred use of low-flexibility polymer films into account, it will be necessary to preform therein the zones where these tubes will be located, a condition that is also met by the thermoforming of the sheets 2 and 3.

As shown in figure 4c, it is conceivable to add flutes 13 to the access routes 8, 9, 12 so as to improve the leaktightness with respect to a tube. Fluted end pieces are thus obtained

(it is also conceivable to structure olive-shaped conical end pieces to achieve the same result).

As shown in figure 4d, it is also conceivable to add an internal reinforcement 14 into the access routes 8, 9, 12, this also making it possible to obtain a perfectly leaktight connection to a tube.

The sheets used are generally of a small thickness so as to provide a minimum level of permeability to gases, in particular to oxygen, and the thermoforming process further reduces this thickness. It is therefore necessary to reinforce the access routes 8, 9, 12 so as to guarantee their solidity and also their leaktightness once they have been connected to a tube.

Reference is now made to figures 5a to 5d, which show various possible shapes of the reliefs 10, 11.

The reliefs 10, 11 may in particular be in the form of corrugations, ripples, notches or burrs, which are shown respectively in figures 5a, b, c and d. The reliefs 10, 11 may form repeating motifs or be irregular. The reliefs 10, 11 may extend over part or all of the bottom 4 of the container 1.

In order to obtain sufficient increases in the culture surface, these reliefs 10, 11 are not produced on a micrometric or nanometric scale but rather at least on a millimetric scale.

As mentioned above, the production of the reliefs 10, 11 on the face of the sheets 2, 3 within the container 1 by thermoforming makes it possible to increase the culture surface for adhering cells.

These reliefs may also be a means of retaining the non-adhering cells on the sheet 2, 3 as the medium is being circulated, in the context of a cell culture with continuous perfusion of medium within the container 1.

In this respect, a relief having a notched shape, shown in figure 5c, said notches being arranged in the interior volume of the container 1, would be particularly suitable for culturing non-adhering cells with perfusion of the medium.

The Applicant has developed the technique of thermoforming for producing the container according to the invention. The container according to the invention is innovative in that it provides a global response to all of the limiting criteria in the use of a flexible pouch for cell culturing. Furthermore, this technology for transforming plastics materials is particularly well suited to the production of a range of cell culture containers, the

dimensional and structural characteristics of which may easily be adapted as a function of the cell types and their applications. Although it is particularly well suited to the preparation of cells for therapeutic purposes, the container according to the invention may also be used for other biotechnological applications that use prokaryotic cell cultures as eukaryotes.

The word 'comprising' or forms of the word 'comprising' as used in this description and in the claims do not limit the invention claimed to exclude any variants or additions.

2003270995 19 Sep 2008

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS:

1. Container intended for the culturing of cells, comprising a first and a second gas-permeable sheet that are secured to one another in the vicinity of their periphery so as to form an interior volume intended to receive the cells, and at least one access route designed to allow the introduction and/or the recovery of the cells, each of said sheets comprising at least one layer made of a polymer material that allows the cells to adhere, wherein at least one of the two sheets is thermoformed.

2. Container according to Claim 1, wherein each sheet is formed essentially of a polymer material that allows the cells to adhere.

3. Container according to Claim 1 or 2, wherein the sheets are secured to one another by welding in the vicinity of their periphery.

4. Container according to any one of Claims 1 to 3, wherein at least a first sheet is thermoformed so as to have, in transverse section, the overall shape of a rectangle comprising a substantially flat base, a side wall and a peripheral wall that forms a rim.

5. Container according to Claim 4, wherein the second sheet is secured to the rim of the first sheet so as to be substantially flat, said second sheet forming the upper wall of the container.

6. Container according to Claim 4, wherein in that the second sheet that forms the upper wall of the container is thermoformed so as to have a geometry which is the same as that of the first sheet, said sheets being secured facing one another by their rims.

7. Container according to any one of Claims 1 to 6, wherein at least one access route communicates with the interior volume of the container through a wall of a thermoformed sheet.

8. Container according to any one of Claims 1 to 7, wherein at least one of the thermoformed sheets has reliefs that are arranged in the interior volume of the container.

9. Container according to Claim 8, wherein the reliefs form repeating or irregular, continuous or separate motifs.

10. System comprising at least two elements connected together as a closed circuit by means of a tube, at least one of said elements being a container according to any one of

Claims 1 to 9, the tube being connected at a first end to the access route of the container and at a second end to another element of the system, so as to allow the cells and/or fluids to pass between the elements of the system.

11. Use of a container according to any one of Claims 1 to 9 or of a system according to Claim 10 for culturing adhering cells and/or cells that are in suspension in the medium.

12. A container substantially as herein described with reference to the accompanying drawings.

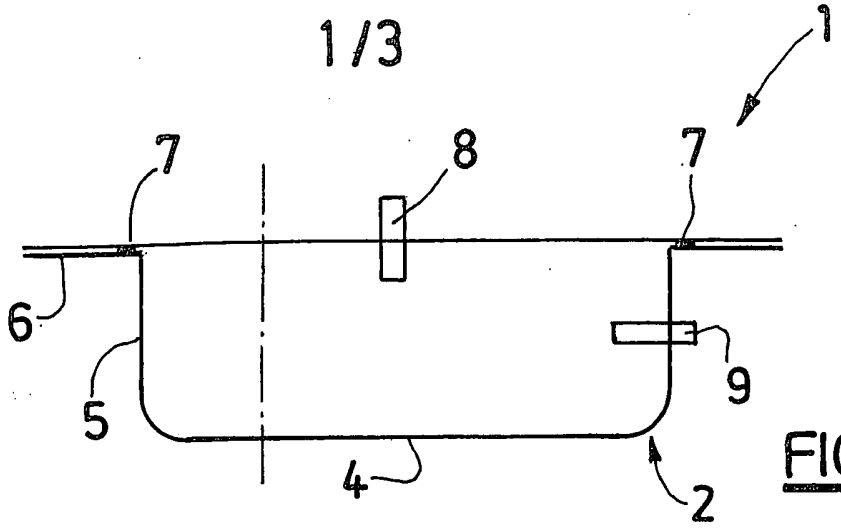

FIG.1

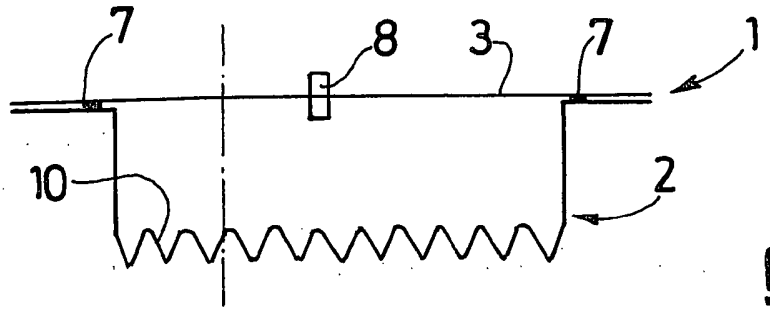

FIG.2a

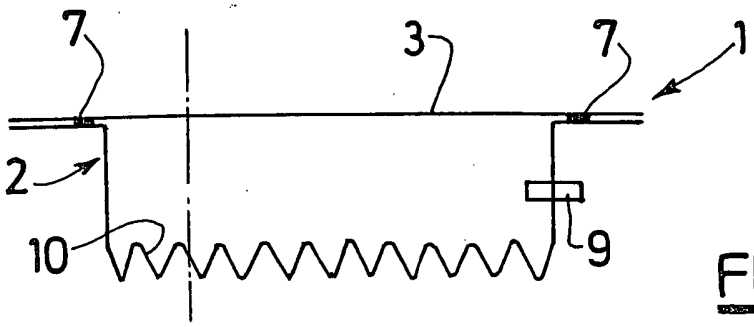

FIG.2b

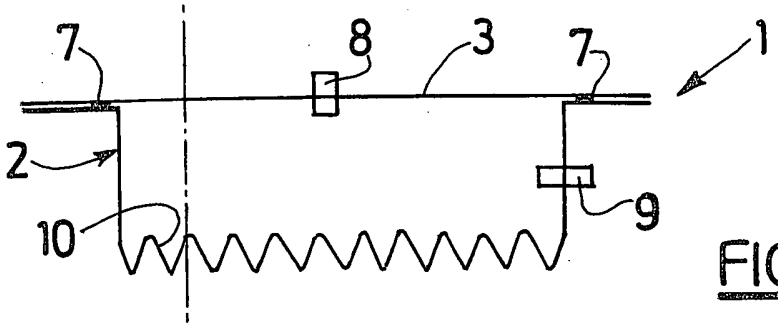

FIG.2c

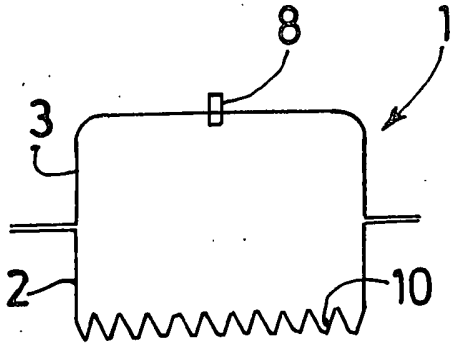

FIG.3a

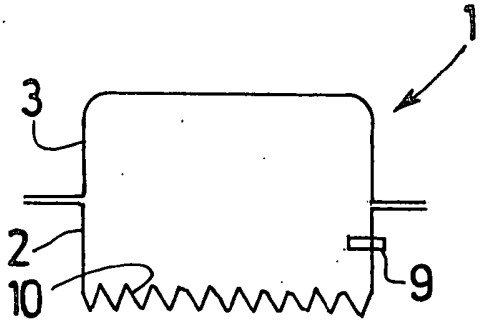

FIG.3b

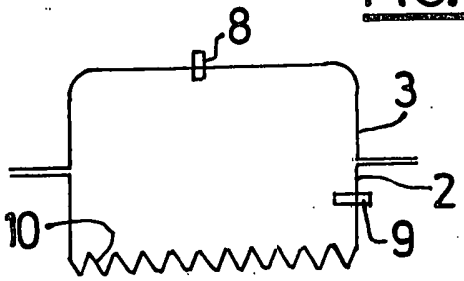

FIG.3c

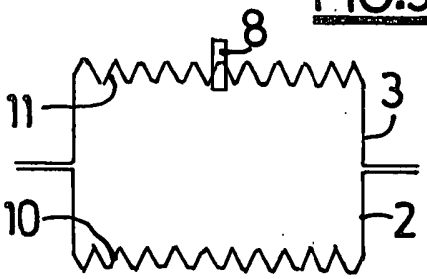

FIG.3d

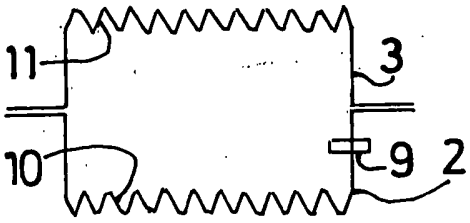

FIG.3e

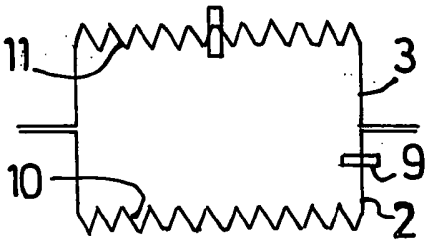

FIG.3f

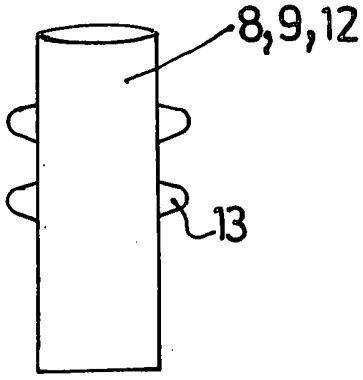

FIG.4c

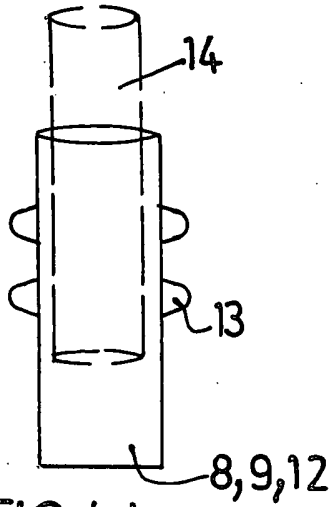

FIG.4d

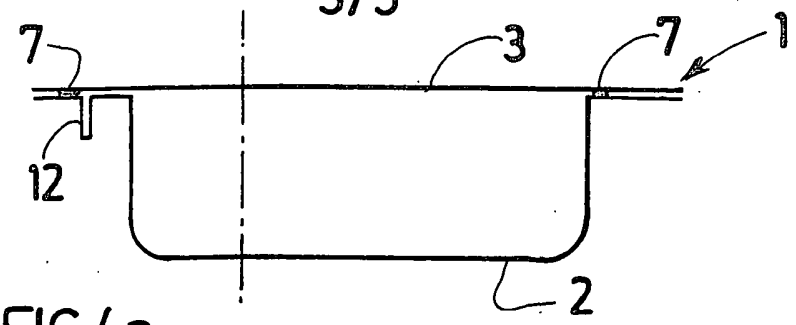

FIG.4a

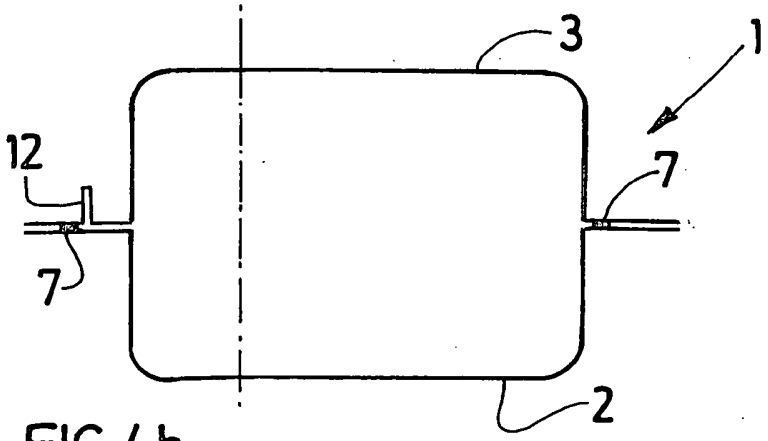

FIG.4b

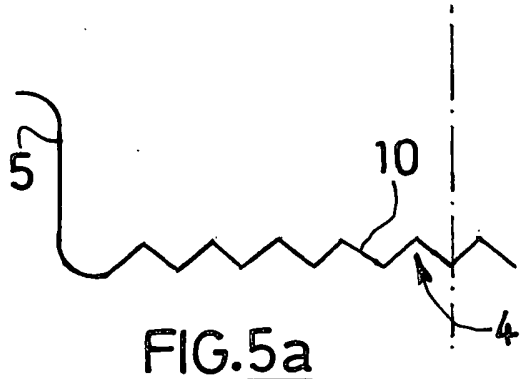

FIG.5a

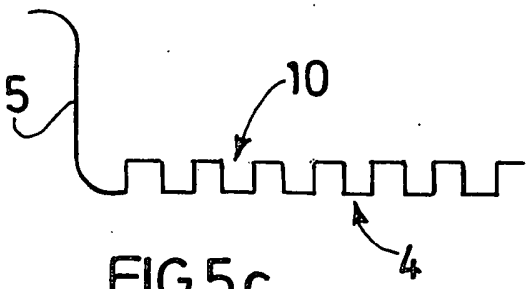

FIG.5c

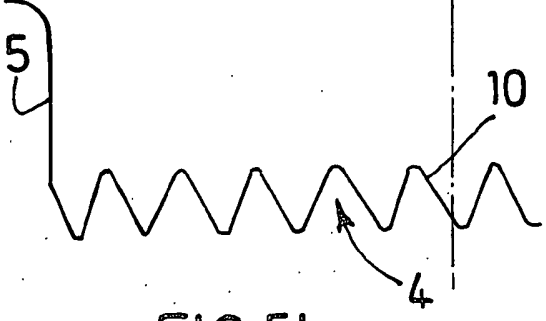

FIG.5b

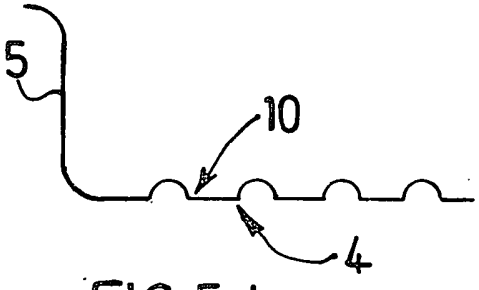

FIG.5d