US009283559B2

(12) **United States Patent**
Berthier et al.

(10) **Patent No.: US 9,283,559 B2**
(45) **Date of Patent: Mar. 15, 2016**

(54) **LID FOR FUNCTIONALIZED MICROFLUIDIC PLATFORM AND METHOD**

(75) Inventors: **Erwin Berthier**, Madison, WI (US); **Peter Cavnar**, Madison, WI (US); **David John Guckenberger**, Oconomowoc, WI (US); **David Beebe**, Monona, WI (US)

(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 323 days.

(21) Appl. No.: **13/467,584**

(22) Filed: **May 9, 2012**

(65) **Prior Publication Data**

US 2013/0302842 A1 Nov. 14, 2013

(51) **Int. Cl.**

C12M 1/34	(2006.01)
C12M 3/00	(2006.01)
B01L 3/00	(2006.01)
G01N 33/50	(2006.01)
A01N 1/02	(2006.01)
B01L 7/00	(2006.01)

(52) **U.S. Cl.**

CPC ***B01L 3/502715*** (2013.01); ***A01N 1/0263*** (2013.01); ***A01N 1/0268*** (2013.01); ***G01N 33/5005*** (2013.01); *B01L 7/50* (2013.01); *B01L 2200/027* (2013.01); *B01L 2300/044* (2013.01); *B01L 2300/047* (2013.01); *B01L 2300/0672* (2013.01); *B01L 2300/0816* (2013.01); *B01L 2300/0887* (2013.01); *B01L 2400/0406* (2013.01); *B01L 2400/0481* (2013.01); *B01L 2400/0677* (2013.01); *B01L 2400/0683* (2013.01); *B01L 2400/0694* (2013.01); *B01L 2400/086* (2013.01)

(58) **Field of Classification Search**

CPC ... A01N 1/0268; B01L 3/502715; B01L 7/50; B01L 7/52; B01L 2200/027; B01L 2300/044; B01L 2300/047; B01L 2300/0672; B01L 2300/0816; B01L 2300/0887; B01L 2400/0406; B01L 2400/0481; B01L 2400/0677; B01L 2400/0694; B01L 2400/086

USPC 435/288.5, 287.2

See application file for complete search history.

(56) **References Cited**

U.S. PATENT DOCUMENTS

2004/0209349	A1 *	10/2004	Goldman et al.	435/287.1
2006/0057629	A1 *	3/2006	Kim	435/6
2006/0205085	A1 *	9/2006	Handique et al.	436/177
2012/0028342	A1 *	2/2012	Ismagilov et al.	435/283.1

OTHER PUBLICATIONS

"SlipChip", Wenbin Du et al, Lab Chip, 2009, 9, 2286-2292.

* cited by examiner

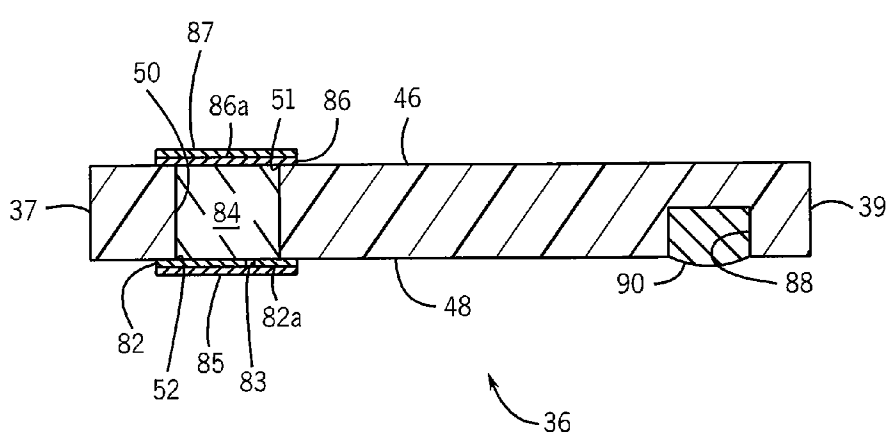
Primary Examiner — Michael Hobbs

(74) *Attorney, Agent, or Firm* — Boyle Fredrickson, S.C.

(57) **ABSTRACT**

A microfluidic device and method is provided for handheld diagnostics and assays. A first substance is frozen in a cryopreservation fluid in a first well of a lid. The lid includes a first surface communicating with a first port of the first well and a second surface communicating with a second port of the first well. A porous membrane is affixed to the first surface so as to overlap the first port and a non-porous membrane is affixed to the second surface so as to overlap the second port. The first substance may be dialytically freed from the cryopreservation fluid at a user desired time. Thereafter, the lid may be moved from a first position wherein the lid is spaced from a base to a second position wherein the lid is adjacent the channel in the base such that the first substance communicates with the input of the channel.

8 Claims, 6 Drawing Sheets



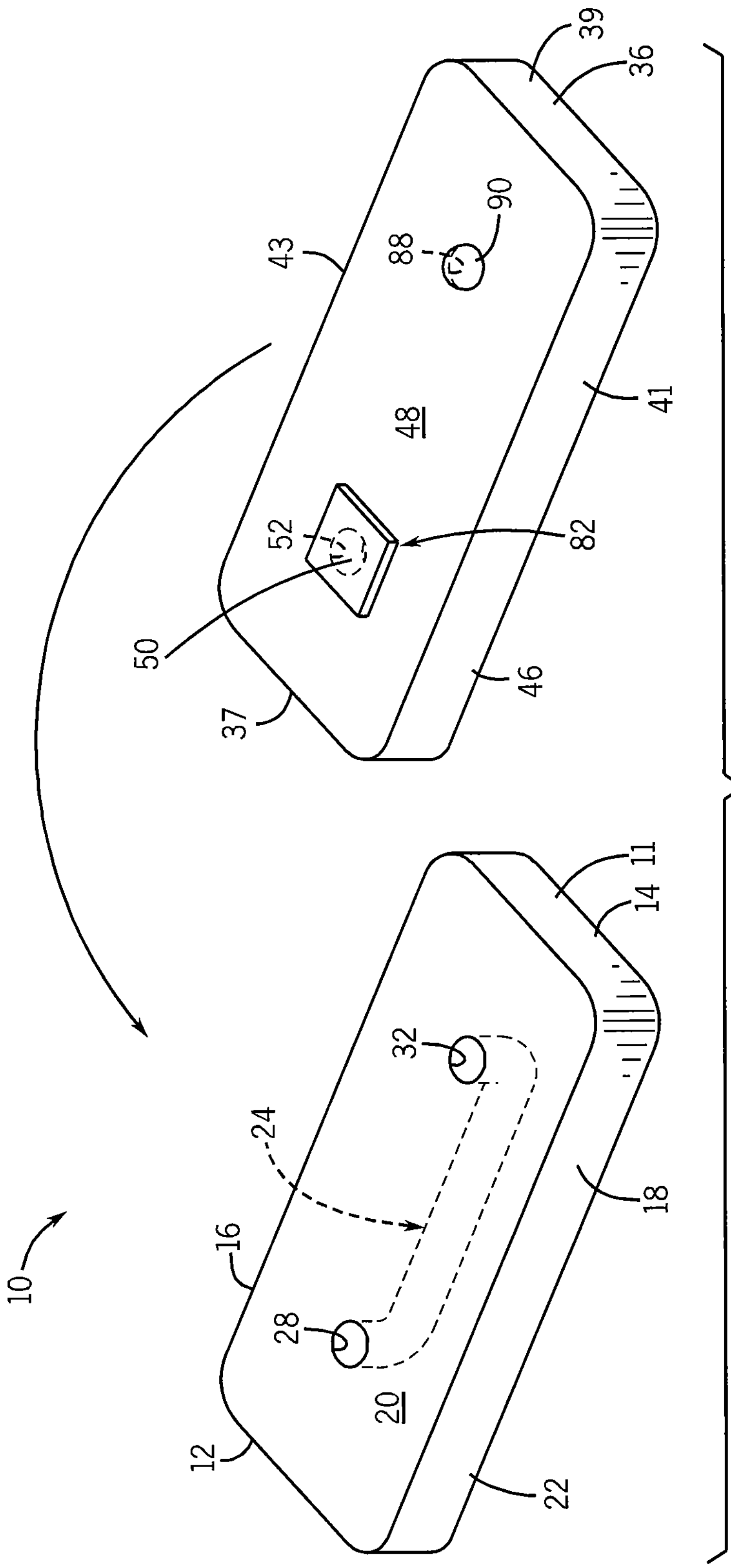


FIG. 1

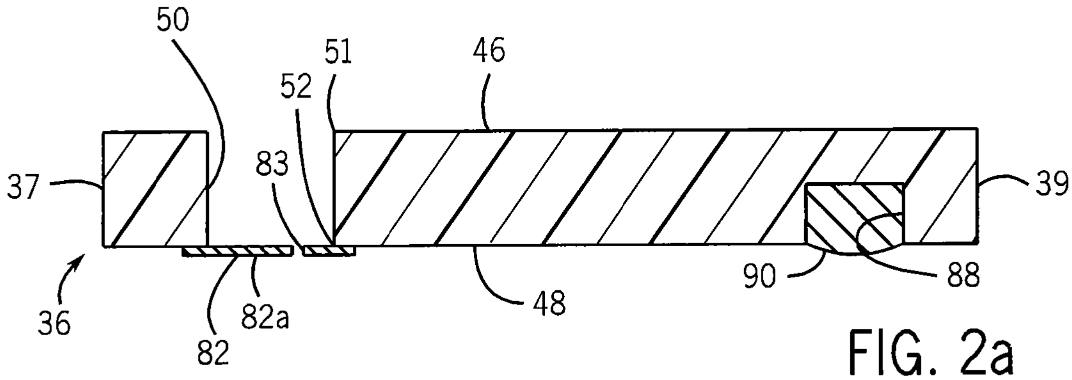


FIG. 2a

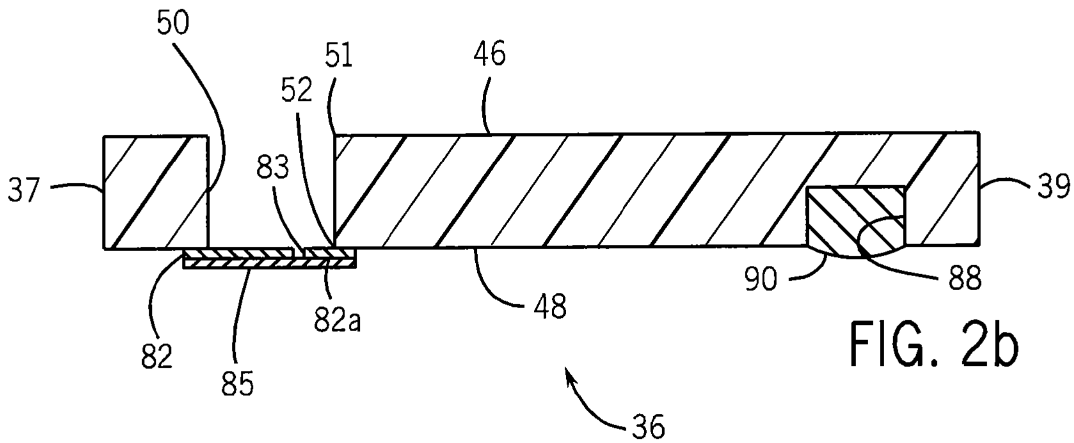


FIG. 2b

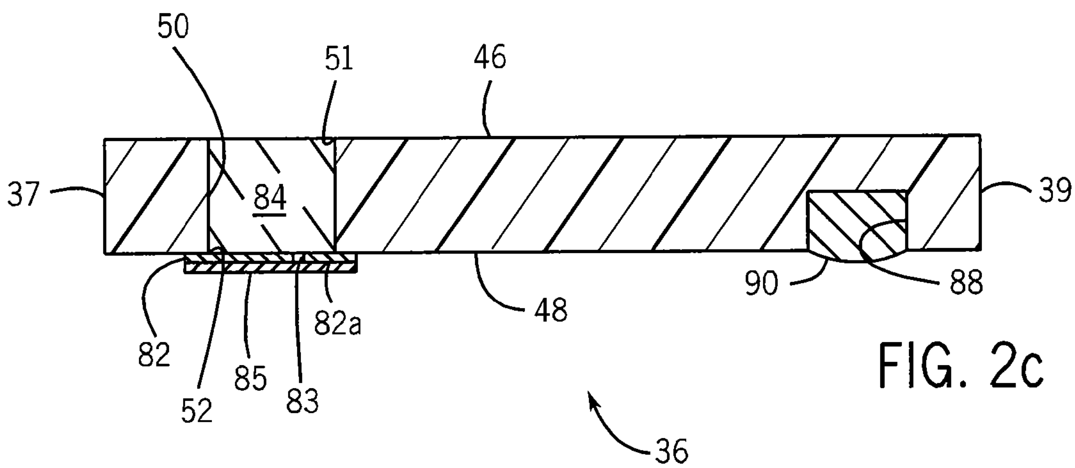


FIG. 2c

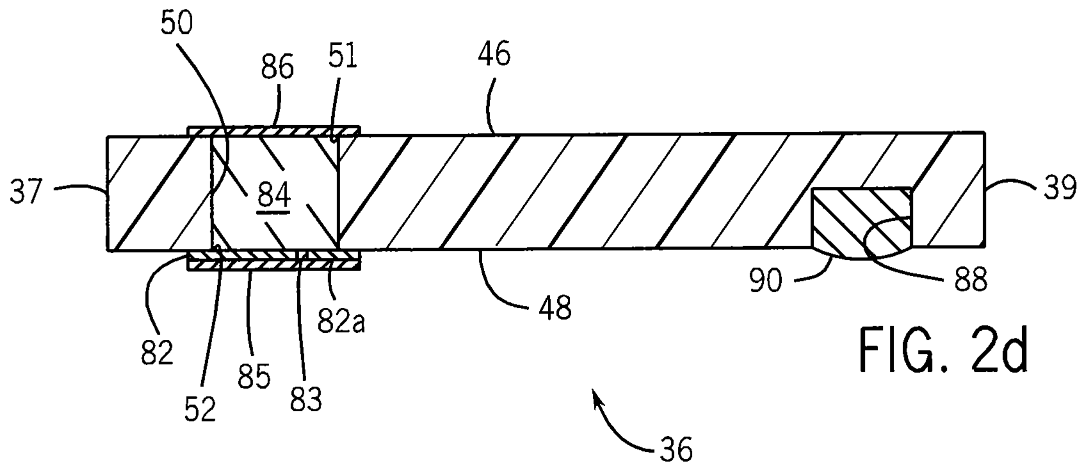


FIG. 2d

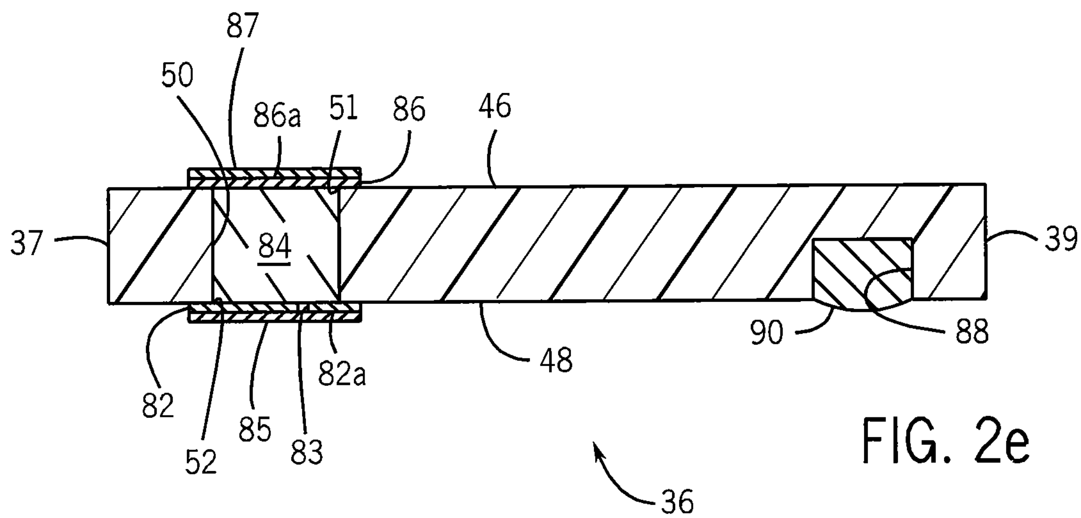


FIG. 2e

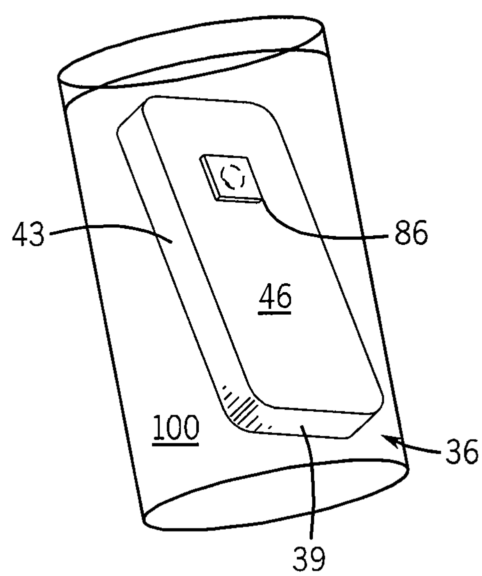


FIG. 3

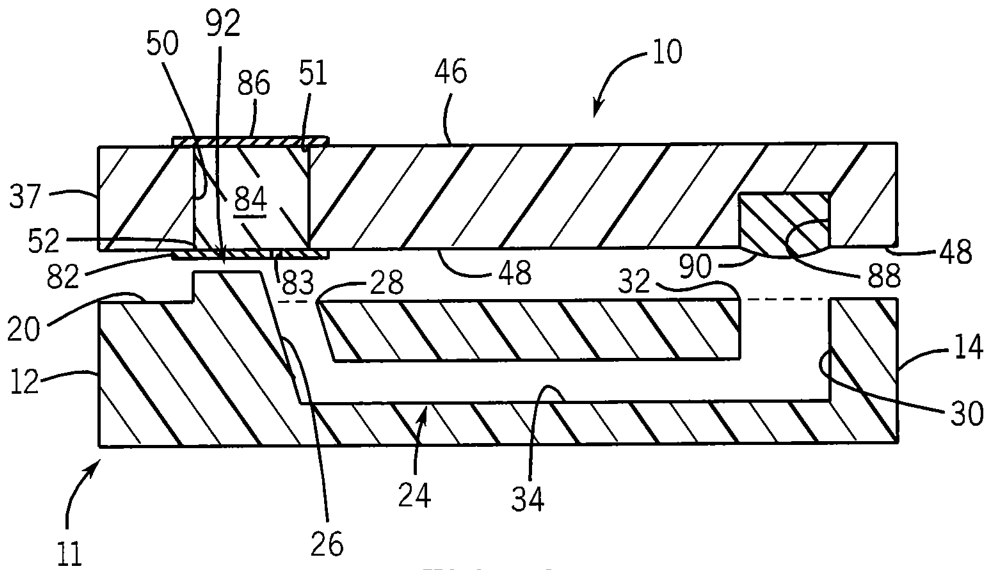

92
50
86
51
46
10
37
84
52
82
83
28
48
32
90
88
48
20
14
12
30
24
34
11
26

FIG. 4

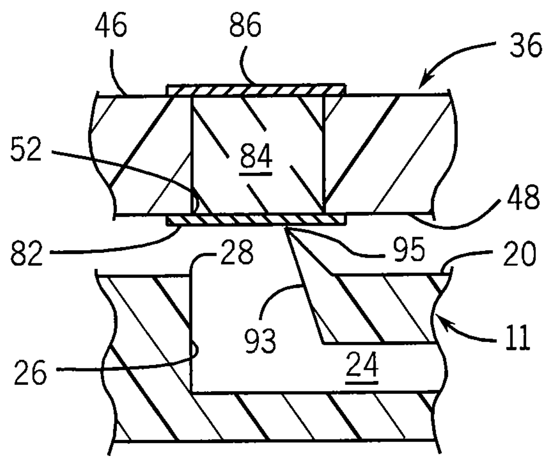
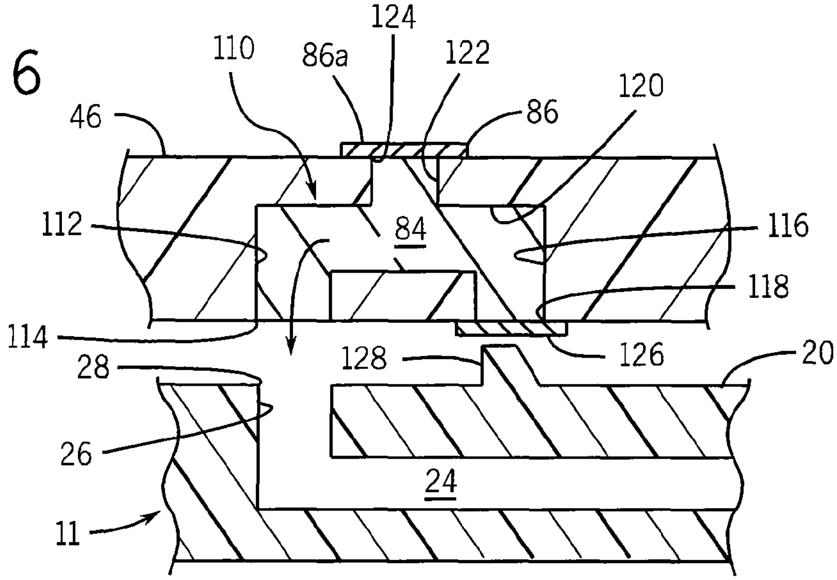

46
86
36
52
84
48
82
28
95
20
93
11
26
24

FIG. 5

FIG. 6



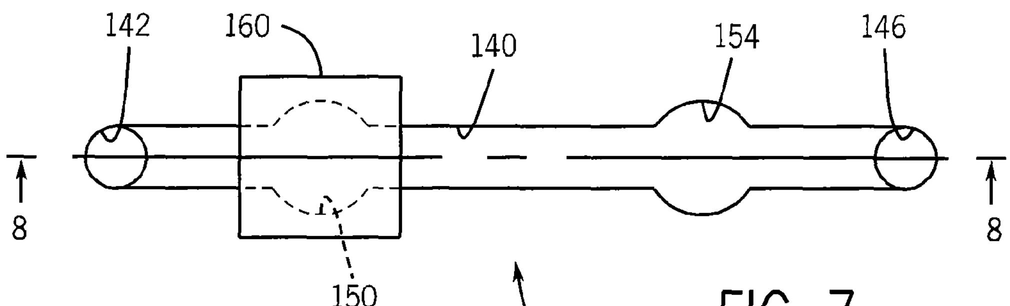


FIG. 7

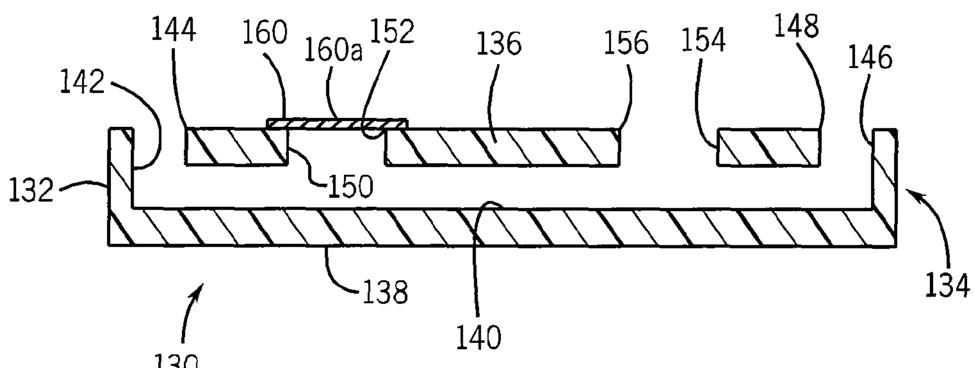


FIG. 8

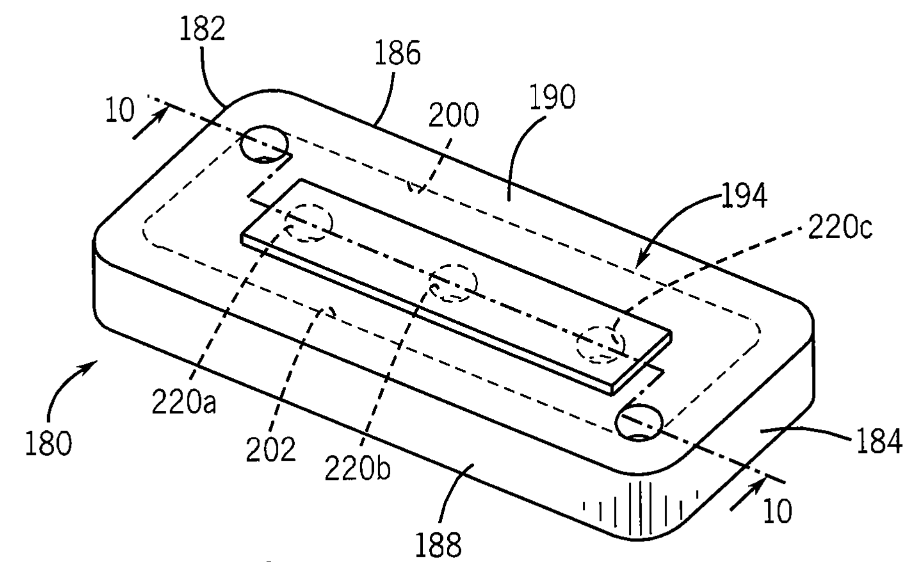

182
186
190
200
10
194
220c
220a
180
202
220b
184
188
10

FIG. 9

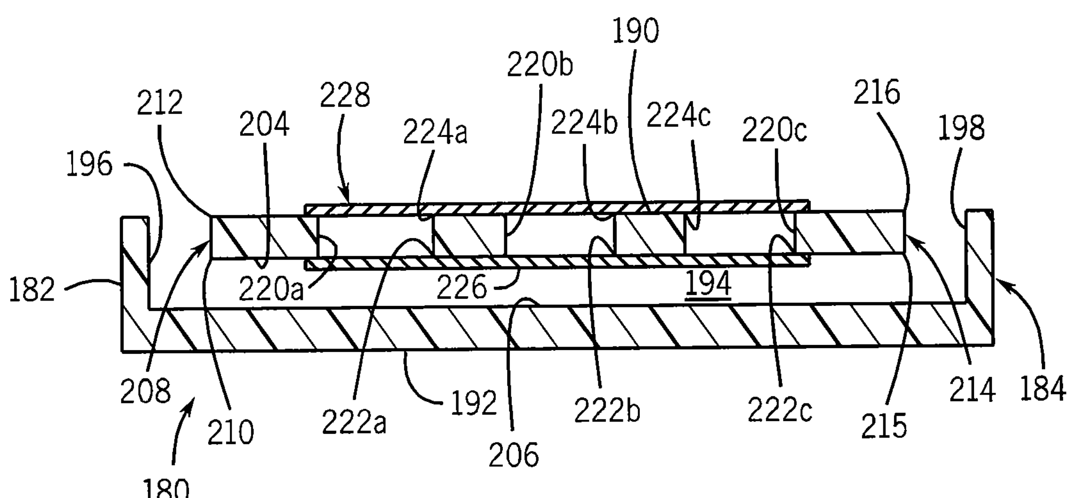

190
220b
212
228
216
204
224a
224b
224c
220c
198
196
182
220a
226
194
208
210
222a
192
222b
222c
214
184
215
206
180

FIG. 10

LID FOR FUNCTIONALIZED MICROFLUIDIC PLATFORM AND METHOD

REFERENCE TO GOVERNMENT GRANT

This invention was made with government support under CA137673 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE INVENTION

This invention relates generally to microfluidic devices, and in particular, to a lid for a functionalized microfluidic platform and method for freezing, storing, shipping, and thawing cell suspensions that maintains their viability and allows for their subsequent culture and study.

BACKGROUND AND SUMMARY OF THE INVENTION

The field of microfluidics has matured significantly over the past two decades. Compelling platforms have been produced to address problems in traditional cell biology techniques that were previously too difficult to solve. Limitations of traditional cell biology techniques have been primarily due to onerous labor requirements and limited spatial and temporal control of the cells' microenvironment. Microfluidics has provided significant efficiency gains by reducing reagent and cell requirements which, in turn, has allowed for high-throughput processing and analysis of a large array of experimental conditions. Microfluidic systems also offer significantly greater control of the cells' microenviroment, such as flow rate, extracellular matrix (ECM) properties, and soluble factor signaling (e.g., forming a chemical gradient in diffusion dominant conditions). However, for microfluidics to make further inroads into cell biology, new microfluidic assays must be cheaper, faster, and in qualitative agreement with techniques traditionally used by biologists. It can be appreciated that microfluidics has tremendous potential to contribute to the development of drug therapies to fight cancer, point-of-care diagnostics for HIV in developing countries, and numerous other applications that are critical to the health and well being of individuals worldwide.

While current microfluidic devices provide a significant improvement in the ability to study fundamental aspects of cell biology, the adoption of microfluidic devices in clinical settings has been slow due to the high level of technicality and external equipment required. For example, current microfluidic assay methods require steps such as washing, flushing, pipetting, and transferring of cells and other materials. As such, most conventional microfluidic devices typically incorporate external elements, such as tubing and syringe pumps, to provide the valving and the mixing functionality necessary to enable an entire assay to be performed within a microfluidic system. These external elements diminish the simplicity and advantages of a microfluidic platform for biological assays.

Further, as is known, the discovery of compounds of interest for therapeutic or research applications is a complex, multitiered process that requires a number of assays of increasing relevance, from biochemical screens and in-vitro live cell assays, to in-vivo animal models. Currently, many laboratories in the areas of natural products purification, microbiology, and toxicology are focused on isolating compounds with putative biological activity for the purpose of discovering new drugs or determining factors of microbial virulence and cytotoxicity. These laboratories have developed low-cost and relatively easy assays to identify compounds of interest through large-scale biochemical screens. In-vitro live-cell assays are an essential step before deciding to pursue costly in-vivo studies, as they: 1) provide more physiologically relevant insight on the biological activity of a compound; 2) are much more time-efficient then prior methodologies; and 3) reduce animal use. However, the accessibility of live-cell assays is limited because they require expensive equipment, a plethora of perishable reagents, and highly trained researchers. These costs and training requirements represent a significant barrier for researchers who are interested in performing an initial in-vitro screening assay. Hence, it is highly desirable to provide a microfluidic platform capable of performing assays, including in-vitro live-cell assays, which do not require any external equipment to operate and which can be adapted to a wide range of situations.

Therefore, it is a primary object and feature of the present invention to provide a functionalized lid for a microfluidic platform and a method for freezing, storing, shipping, and thawing cell suspensions in a manner that maintains their viability and allows their subsequent culture and study.

It is a further object and feature of the present invention to provide a functionalized lid for a microfluidic platform and a method for freezing, storing, shipping, and thawing cell suspensions which do not require any external equipment to operate and which can be adapted to a wide range of situations.

It is a still further object and feature of the present invention to provide a microfluidic platform and a method for freezing, storing, shipping, and thawing cell suspensions which are simple to implement and inexpensive to manufacture.

In accordance with the present invention, a lid is provided for a microfluidic platform. The microfluidic platform includes a base having outer surface and a channel therethough for receiving fluid therein. The channel has input and output ports communicating with the outer surface. The lid includes a body having a first well extending therethrough. The first well includes first and second ports communicating therewith and is adapted for receiving a first substance therein. A first membrane extends over the first port of the lid. The first membrane retains the first substance in the first well and allows a second substance to diffuse therethrough. A non-porous second membrane extends over the second port. The second membrane preventing the second substance form diffusing therethrough.

The body may include first and second spaced surfaces. The first port communicates with the first surface and the second port communicates with the second surface. The first and second surfaces may be planar. The first surface lies in a first plane and the second surface lies in a second plane generally parallel to the first plane. An absorbent may be provided adjacent the second surface and a removable seal may overlap the second membrane for isolating the first substance in the first well. The lid is movable between a first position wherein the lid is spaced from the base to a second position wherein the lid is adjacent the channel such that the first substance communicates with the input of the channel.

In accordance with a further aspect of the present invention, a method is provided for handheld diagnostics and assays. The method includes the step of capturing a first substance in a cryopreservation fluid in a first well of a lid. The lid includes first and second ports communicating with the first well. The first substance is dialytically freeing from the cryopreservation fluid.

In addition, it is contemplated to provide a channel having an input and an output in a base. The lid is moved from a first position wherein the lid is spaced from the base to a second position wherein the lid is adjacent the channel such that the first substance communicates with the input of the channel. A first membrane may be provided over the first port of the lid. The first membrane retains the first substance in the first well and allowing a dialysis fluid to diffuse therethrough. A second membrane may be provided over the second port. The second membrane prevents a dialysis fluid from diffusing therethrough.

A channel having an input and an output in a base may be provided. The lid may be moved from a first position wherein the lid is spaced from the base to a second position wherein the lid is adjacent the channel such that the first substance communicates with the input of the channel. The first substance may be drawn through the channel, e.g. by positioning an absorbent in communication with the output of the channel.

In accordance with a still further aspect of the present invention, a method is provided from for handheld diagnostics and assays. The method includes the step of freezing a first substance in a cryopreservation fluid in a first well of a lid. The lid includes a first surface communicating with a first port of the first well and a second surface communicating with a second port of the first well. A porous membrane is affixed to the first surface so as to overlap the first port and a non-porous membrane is affixed to the second surface so as to overlap the second port. The first substance is dialytically freed from the cryopreservation fluid.

The method may further include the step of providing a channel having an input and an output in a base. The lid may be moved from a first position wherein the lid is spaced from the base to a second position wherein the lid is adjacent the channel such that the first substance communicates with the input of the channel. The first substance may be drawn through the channel, e.g. by positioning an absorbent in communication with the output of the channel. A seal may be removed from the non-porous membrane so as to allow the first substance to pass therethrough. Alternatively, the non-porous membrane may be pierced so as to allow the first substance to pass therethrough.

It is intended for the porous membrane to retain the first substance in the first well and to allow a dialysis fluid to diffuse therethrough. On the other hand, the non-porous membrane prevents the dialysis fluid from diffusing therethrough. The step of dialytically freeing the first substance from the cryopreservation fluid includes the step of depositing the lid in a dialysis fluid for a time period.

BRIEF DESCRIPTION OF THE DRAWINGS

The drawings furnished herewith illustrate a preferred construction of the present invention in which the above advantages and features are clearly disclosed as well as other which will be readily understood from the following description of the illustrated embodiment.

In the drawings:

FIG. **1** is an exploded, isometric view of a microfluidic device in accordance with the present invention;

FIG. **2***a* is a cross sectional view of a lid of the microfluidic device of FIG. **1** in accordance with a first step of the methodology of the present invention;

FIG. **2***b* is a cross sectional view of a lid of FIG. **2***a* in accordance with a second step of the methodology of the present invention;

FIG. **2***c* is a cross sectional view of a lid of FIG. **2***b* in accordance with a third step of the methodology of the present invention;

FIG. **2***d* is a cross sectional view of a lid of FIG. **2***c* in accordance with a fourth step of the methodology of the present invention;

FIG. **2***e* is a cross sectional view of a lid of FIG. **2***d* in accordance with a fifth step of the methodology of the present invention;

FIG. **3** is an isometric view of a lid of the microfluidic device of FIG. **1** in accordance with a sixth step of the methodology of the present invention;

FIG. **4** is an enlarged, cross sectional view showing a microfluidic device for effectuating the methodology of the present invention;

FIG. **5** is an enlarged, cross sectional view showing an alternate embodiment of a microfluidic device for effectuating the methodology of the present invention;

FIG. **6** is an enlarged, cross sectional view showing a further alternate embodiment of a microfluidic device for effectuating the methodology of the present invention;

FIG. **7** is schematic, top plan view of a still further alternate embodiment of a microfluidic device for effectuating the methodology of the present invention;

FIG. **8** is a cross-sectional view of the microfluidic device of the present invention take along line **8-8** of FIG. **7**;

FIG. **9** is an isometric view of a still further embodiment of a lid for a microfluidic device in accordance with the present invention;

FIG. **10** is a cross sectional view of the lid of the microfluidic device of the present invention taken along line **10-10** of FIG. **9**.

DETAILED DESCRIPTION OF THE DRAWINGS

Referring to FIGS. **1-4**, a microfluidic device for performing a methodology in accordance with the present invention is generally designated by the reference numeral **10**. Microfluidic device **10** may be formed from polystyrene (PS), however, other materials are contemplated as being within the scope of the present invention. In the depicted embodiment, microfluidic device **10** includes base **11** having first and second ends **12** and **14**, respectively; first and second sides **16** and **18**, respectively; and upper and lower surfaces **20** and **22**, respectively. Channel **24** extends through base **11** of microfluidic device **10** and includes a first vertical portion **26** terminating at an input port **28** that communicates with upper surface **20** of base **11** of microfluidic device **10** and a second vertical portion **30** terminating at an output port **32** also communicating with upper surface **20** of base **11** of microfluidic device **10**. First and second vertical portions **26** and **30**, respectively, of channel **24** are interconnected by and communicate with horizontal portion **34** of channel **24**. The dimension of channel **34** connecting input port **28** and output port **32** is arbitrary.

Microfluidic device **10** further includes lid **36** having first and second ends **37** and **39**, respectively; first and second sides **41** and **43**, respectively; and upper and lower surfaces **46** and **48**, respectively. Similar to base **11**, lid **36** may be formed from polystyrene (PS), however, other materials are contemplated as being within the scope of the present invention. As best seen in FIGS. **2***a*-**2***e*, lid **36** further includes first well **50** extending therethrough. First well **50** has a first end terminating at first port **51** that communicates with upper surface **46** and a second end terminating at second port **52** that communicates with lower surface **48**. The diameter of second port **52** is generally equal to the diameter of input port **28** in base **11**. It can be appreciated that while first and second ports **51** and **52**, respectively, are depicted as being on opposites surfaces of lid **36**, first and second ports **51** and **52**, respectively, may communicate with a single surface or other surfaces of lid **36** without deviating from the scope of the present invention.

Non-porous membrane **82** overlaps second port **52** of first well **50** of lid **36** and is bonded to lower surface **48** thereof. It is contemplated for non-porous membrane **82** to include hole **83** therethrough and for first seal **85** to be removably bonded to outer surface **82***a* of non-porous membrane **82** and/or lower surface **48** of lid **36**, FIG. **2***b*, to hermetically isolate the interior of first well **50**, as hereinafter described. Lid **36** further includes second well **88** formed in lower surface **48** thereof. As hereinafter described, in order to facilitate fluid flow in channel **24**, it is contemplated to provide absorbent **90** in second well **88**.

In operation, it is contemplated to utilize microfluidic device **10** to perform one or a series of steps of a desired assay, FIG. **2***c*. More specifically, first well **50** of lid **36** is loaded with a desired substance **84**, such as cells and reagents, along with a cryopreservation fluid, e.g. dimethyl sulfoxide (DMSO). The cryopreservation fluid is added to substance **84** during the freezing process, hereinafter described, to prevent crystallization and cell death. Porous membrane **86** is bonded to upper surface **48** of lid **36** so as to overlap first port **51**, FIG. **2***d*, and second seal **87** is removably bonded to outer surface **86***a* of porous membrane **86** and/or upper surface **46** of lid **36** to hermetically isolate the interior of first well **50**, FIG. **2***e*. As described, substance **84** and the cryopreservation fluid in first well **50** is captured between non-porous membrane **82** and porous membrane **86**. For reasons hereinafter described, it is intended for porous membrane **86** to includes micropores therein having pore sizes in a desired range, i.e., from 0.1 μm to 3 μm, so as to allow a predetermined fluid to pass therethrough, but retain substance **84** in first well **50**. With substance **84** and the cryopreservation fluid captured in first well **50**, substance **84** and the cryopreservation fluid may be frozen in accordance with established procedures in first well **50** of lid **36** for better packaging, storage and shipping.

In order to flow substance **84** into channel **24** through base **11** of microfluidic device **10**, substance **84** must be freed from the cryopreservation fluid. By way of example, second seal **87** is removed from lid **36** so as to expose porous membrane **86**. Thereafter, lid **36** is inserted into a bath of dialysis fluid **100**, e.g. cell culture media, for a time period, e.g. two to five minutes, FIG. **3**. Dialysis fluid **100** diffuses through porous membrane **86** and frees substance **84** from the cryopreservation fluid. Once substance **84** is freed, as heretofore described, channel **24** is filled with a predetermined fluid and first seal **85** is removed from lid **36** such that hole **83** is exposed. Alternatively, first seal **85** and non-porous membrane **82** may be pieced so as to allow substance **84** to flow therethrough. Lid **36** is then positioned on base **11** such that: 1) lower surface **48** of lid **36** is bought into contact with or adjacent to upper surface **20** of base **11**; 2) second port **52** in lid **36** is aligned with and brought into close proximity with input port **28** in base **11** such that substance **84** is in fluid communication with channel **24** through hole **83**; and 3) absorbent **90** contacts the fluid in channel **24** at output port **32** such that fluid flow within channel **24** is induced. Alternatively, in order to induce fluid flow in channel **24**, absorbent **90** in second well **88** may be removed and an input of a capillary (not shown) may be provided in communication with second well **88**. The output of the capillary may operatively connected to a pumping mechanism (not shown).

Referring to FIGS. **4-6**, various embodiments are depicted to urge substance **84** from first well **50** of lid **36** and into channel **24**. By way of example, post **92** may project from upper surface **20** of base **11**, FIG. **4**. As lid **36** is positioned on base **11**, it is contemplated for post **92** projecting from upper surface **20** of base **11** to be urged against non-porous membrane **82** and into first well **50**. It can be appreciated that as post **92** is urged against non-porous membrane **82**, substance **84** is urged from first well **50** and into channel **24** through hole **83** in non-porous membrane **82**.

Alternatively, non-porous membrane **82** may be provided without hole **83** therein. As such, it is contemplated for protrusion **93** having a piercing tip **95** to project from upper surface **20** of base **11**, FIG. **5**. As lid **36** is positioned on base **11**, piercing tip **95** of protrusion **93** projecting from upper surface **20** of base **11** engages and ruptures non-porous membrane **82**. Thereafter, as protrusion **93** enters first well **50**, substance **84** is urged from first well **50** and into channel **24** through: 1) the newly created hole in non-porous membrane **82**; and 2) second port **52** of first well **50** of lid **36**.

Once the entirety of substance **84** in first well **50** of lid **36** flows and/or is urged into channel **24**, lid **36** may be removed from base **11** of microfluidic device **10** and discarded. Thereafter, additional steps of an assay may be performed by sequentially positioning additional lids **36**, as heretofore described, on base **11**, thereby triggering operation of microfluidic device **10**.

Referring to FIG. **6**, it is contemplated for first well **50** in lid **36** to be defined by channel **110** extending therethrough. Channel **110** includes a first vertical portion **112** terminating at port **114** that communicates with lower surface **48** of lid **36** of microfluidic device **10** and a second vertical portion **116** terminating at port **118** also communicating with lower surface **48** of lid **36** of microfluidic device **10**. First and second vertical portions **112** and **116**, respectively, of channel **110** are interconnected by and communicate with horizontal portion **120** of channel **110**. Channel **110** also includes a third vertical portion **122** having a first end communicating with horizontal portion **120** of channel **110** and a second end terminating at port **124** communicating with upper surface **46** of lid **36** of microfluidic device **10**.

In operation, non-porous membranes, e.g. membrane **126**, are bonded to lower surface **48** of lid **36** so as to overlap ports **114** and **118** therein. First well **50** of lid **36** is loaded with a desired substance **84**, such as cells and reagents, along with a cryopreservation fluid, e.g. dimethyl sulfoxide (DMSO). The cryopreservation fluid is added to substance **84** during the freezing process, hereinafter described, to prevent crystallization and cell death. Porous membrane **86** is bonded to upper surface **48** of lid **36** so as to overlap port **124** and a seal is removably bonded to outer surface **86***a* of porous membrane **86** and/or upper surface **46** of lid **36** to hermetically isolate the interior of first well **50**, as heretofore described.

As described, substance **84** and the cryopreservation fluid in first well **50** is captured between the non-porous membranes over ports **114** and **118** and porous membrane **86**. For reasons hereinafter described, it is intended for porous membrane **86** to includes micropores therein having pore sizes in a desired range, i.e., from 0.1 μm to 3 μm, so as to allow a predetermined fluid to pass therethrough, but retain substance **84** in first well **50**. With substance **84** and the cryopreservation fluid captured in first well **50**, substance **84** and the cryopreservation fluid may be frozen in accordance with established procedures in first well **50** of lid **36** for better packaging, storage and shipping.

In order to flow substance **84** into channel **24** through base **11** of microfluidic device **10**, substance **84** must be freed from the cryopreservation fluid. By way of example, the seal is removed from lid **36** so as to expose porous membrane **86**. Thereafter, lid **36** is inserted into a bath of dialysis fluid **100**, e.g. cell culture media, for a time period, e.g. two to five minutes, FIG. **3**. Dialysis fluid **100** diffuses through porous membrane **86** and frees substance **84** from the cryopreservation fluid. Once substance **84** is freed, as heretofore described, channel **24** is filled with a predetermined fluid and the non-porous membrane over port **114** is removed from lid **36** such that port **114** is exposed. Lid **36** is then positioned on base **11** such that: 1) lower surface **48** of lid **36** is bought into contact with or adjacent to upper surface **20** of base **11**; and 2) port **114** in lid **36** is aligned with and brought into close proximity with input port **28** in base **11** such that substance **84** is in fluid communication with channel **24**. In order to urge substance **84** from first well **50** of lid **36** and into channel **24**, post **128** projecting from upper surface **20** of base **11** is urged against non-porous membrane **126** and into second vertical portion **116** of channel **110**. It can be appreciated that as post **128** is urged against non-porous membrane **126**, substance **84** is urged from channel **110** of first well **50** and into channel **24** through port **114**.

As previously explained, once the entirety of substance **84** in first well **50** of lid **36** flows and/or is urged into channel **24**, lid **36** may be removed from base **11** of microfluidic device **10** and discarded. Thereafter, additional steps of an assay may be performed by sequentially positioning additional lids **36**, as heretofore described, on base **11**, thereby triggering operation of microfluidic device **10**.

Referring to FIGS. **7-8**, a further embodiment of a microfluidic device for performing a methodology in accordance with the present invention is generally designated by the reference numeral **130**. Microfluidic device **130** may be formed from polystyrene (PS), however, other materials are contemplated as being within the scope of the present invention. In the depicted embodiment, microfluidic device **130** has first and second ends **132** and **134**, respectively; and upper and lower surfaces **136** and **138**, respectively. Channel **140** extends through microfluidic device **10** and includes a first vertical portion **142** terminating at an input port **144** that communicates with upper surface **136** of microfluidic device **130** and a second vertical portion **146** terminating at an output port **148** also communicating with upper surface **136** of microfluidic device **10**.

Microfluidic device **130** may also include first well **150** having a first end communicating with channel **140** and a second end terminating at port **152** communicating with upper surface **136** of microfluidic device **130**. Second well **154** is spaced from first well **150** and has a first end communicating with channel **140** and a second end terminating at port **156** communicating with upper surface **136** of microfluidic device **130**.

In operation, first well **150** of microfluidic device **130** is loaded with a desired substance, such as cells and reagents, along with a cryopreservation fluid, e.g. dimethyl sulfoxide (DMSO). A blockage (not shown) may be providing in channel **140** to retain the desired substance and cryopreservation fluid combination in first well **150**. Alternatively, the walls defining channel **140** adjacent the first end of first well **150** may include a hydrophobic pattern for prevent the desired substance and cryopreservation fluid combination from flowing through channel **140** except if a certain pressure threshold is reached. Thereafter, porous membrane **160** is bonded to upper surface **136** of microfluidic device **130** so as to overlap port **152** and a seal is removably bonded to outer surface **160***a* of porous membrane **160***a* to upper surface **136** of microfluidic device **130** to isolate the interior of first well **150**, as heretofore described.

As described, the substance and the cryopreservation fluid combination is captured in first well **150** adjacent porous membrane **160**. For reasons hereinafter described, it is intended for porous membrane **160** to includes micropores therein having pore sizes in a desired range, i.e., from 0.1 μm to 3 μm, so as to allow a predetermined fluid to pass therethrough, but retain the substance in first well **150**. With the substance and the cryopreservation fluid combination captured in first well **150**, the substance and the cryopreservation fluid combination may be frozen in accordance with established procedures in first well **150** of microfluidic device **130** for better packaging, storage and shipping.

In order to flow the substance into channel **140** of microfluidic device **130**, the substance must be freed from the cryopreservation fluid. By way of example, the seal is removed from upper surface **136** of microfluidic device **10** so as to expose porous membrane **160**. Thereafter, microfluidic device **130** may be inserted into a bath of dialysis fluid, e.g. cell culture media, for a time period, e.g. two to five minutes. The dialysis fluid diffuses through porous membrane **160** and frees the substance from the cryopreservation fluid.

Once the substance is freed, as heretofore described, the blockage (if present) may be removed from channel **140** and channel **140** may be filled with a predetermined fluid. In addition, a cell culture may be provided in second well **154**. Fluid flow may be generated in channel **140** in any conventional matter, such as by passive pumping or by positioning an absorbent at output port **148** of channel **140**. The fluid flow in channel **140** may be used to carry the substance from first well **150** into communication with the cell culture in second well **154** for processing or the like.

An additional contemplated application of the present invention is to provide a kit incorporating microfluidic device **10** wherein an end user can preload biomaterial of choice (cells, tissues, etc) in channel **24** of base **11** using a first lid **36**. Thereafter, a series of additional lids may be provided in the kit for acting on the biomaterial in channel **24**. For example, the series of lids may be used for a variety of purposes, such as gradient chemotaxis; to contain the biomaterial; and/or for drug treatment. After the end user manipulates the biomaterial as desired, a series of additional lids may be provided that allow the end user to complete an entire immunostaining protocol without the need for pipettes. These lids would contain liquids, including the antibodies and fluorophores, needed for detection. The end user would effectuate the protocol by applying the lids, as heretofore described, in a specified sequence. This application allows for higher throughput, cheaper costs, and faster protocol times.

It can be appreciated that lid **36** may include an array of first and second well combinations, as heretofore described. Likewise, base **11** may including an array of channels **24**, as heretofore described. As a result, it is contemplated for a plurality of assays to be simultaneously conducted using a single base and lid combination, without deviating from the scope of the present invention.

Referring to FIGS. **9-10**, an alternate embodiment of a lid for effectuating the methodology of the present invention is generally designated by the reference numeral **180**. Lid **180** is defined by first and second ends **182** and **184**, respectively; first and second sides **186** and **188**, respectively; and upper and lower surfaces **190** and **192**, respectively. It is intended for lid **180** to be formed from polystyrene (PS), however, other materials are contemplated as being within the scope of the present invention.

Lid **180** further includes chamber **194** therein. In the depicted embodiment, chamber **194** is defined by first and second end walls **196** and **198**, respectively; first and second side walls **200** and **202**, respectively; and upper and lower surfaces **204** and **206**, respectively. As best seen in FIG. **10**, upper surface **204** is spaced from and generally parallel to upper surface **190** of lid **180** and lower surface **206** is spaced from and generally parallel to lower surface **192** of lid **180**. First vertical channel **208** has a lower end **210** communicating with upper surface **204** defining chamber **194** and an upper end defining an input port **212** that communicates with upper surface **190** of lid **180**. Second vertical channel **214** has a lower end **215** communicating with upper surface **204** defining chamber **194** and an upper end defining an output port **216** that communicates with upper surface **190** of lid **180**.

Lid **180** also includes a plurality of axially spaced wells **220***a*-**220***c* having corresponding lower ends **222***a*-**222***c* communicating with chamber **194** and corresponding upper ends **224***a*-**224***c* communicating with upper surface **190** of lid **180**. Porous membrane **226** is bonded to upper surface **204** defining chamber **194** so as to overlap lower ends **222***a*-**222***c*. For reasons hereinafter described, it is intended for porous membrane **226** to includes micropores therein having pore sizes in a desired range, i.e., from 0.1 μm to 3 μm, so as to allow a predetermined fluid to pass therethrough.

In operation, wells **220***a*-**220***c* are loaded with a desired substance **84**, such as cells and reagents as heretofore described, along with a cryopreservation fluid, e.g. dimethyl sulfoxide (DMSO). The cryopreservation fluid is added to substance **84** during the freezing process, hereinafter described, to prevent crystallization and cell death. Non-porous membrane **228** overlaps upper ends **224***a*-**224***c* of wells **220***a*-**220***c*, respectively, and is removeably bonded to upper surface **190** of lid **180** to the interior of wells **220***a*-**220***c*. As described, substance **84** and the cryopreservation fluid in wells **220***a*-**220***c* is captured between non-porous membrane **228** and porous membrane **226**. With substance **84** and the cryopreservation fluid captured in wells **220***a*-**220***c*, substance **84** and the cryopreservation fluid may be frozen in accordance with established procedures for better packaging, storage and shipping.

In order to free substance **84** from the cryopreservation fluid, dialysis fluid, e.g. cell culture media, is flowed into chamber **194** through input port **212** so as to fill chamber **194** therewith. The dialysis fluid is maintained in chamber **194** for a time period, e.g. two to five minutes. It is intended for the dialysis fluid to diffuse through porous membrane **226** and free substance **84** from the cryopreservation fluid. Once substance **84** is freed, as heretofore described, the dialysis fluid is removed from chamber **194** through output port **216**. Thereafter, non-porous membrane **228** may be removed from lid **180** such that wells **220***a*-**220***c* are exposed. Alternatively, non-porous membrane **228** may be pieced so as to allow substance **84** to flow therethrough. Lid **180** may be then positioned on an appropriate base, as heretofore described, such that substance **84** in wells **220***a*-**220***c* are in fluid communication with channel(s) in the base. Fluid flow from wells **220***a*-**220***c* may be induced in any manner, as heretofore described.

Various modes of carrying out the invention are contemplated as being within the scope of the following claims particularly pointing out and distinctly claiming the subject matter that is regarded as the invention.

We claim:

1. A lid, for a functionalized microfluidic platform, the microfluidic platform including a base having outer surface and a channel therethough for receiving fluid therein, the channel having input and output ports communicating with the outer surface, comprising:

a body having a first well extending therethrough, the first well including first and second ports communicating therewith and being adapted for receiving a first substance therein;

a first membrane extending over the first port of the lid, the first membrane retaining the first substance in the first well and allowing a second substance to diffuse therethrough into the first well;

a removable seal releaseably connected to at least one of the first membrane and the body adjacent the first membrane, the seal moveable between a first position wherein the removable seal overlaps and engages the first membrane and hermetically isolates the first well and a second position wherein the removable seal is spaced from the membrane so as to allow the second substance to diffuse through the first membrane; and

a non-porous second membrane extending over the second port, the second membrane preventing the first substance and the second substance from diffusing therethrough.

2. The lid of claim **1** wherein body includes first and second spaced surfaces, the first port communicating with the first surface and the second port communicating with the second surface.

3. The lid of claim **2** wherein the first and second surfaces are planar.

4. The lid of claim **2** wherein the first surface lies in a first plane and the second surface lies in a second plane generally parallel to the first plane.

5. The lid of claim **2** further comprising an absorbent adjacent the second surface.

6. The lid of claim **1** wherein the second membrane includes a hole therethrough.

7. The lid of claim **1** wherein the lid is movable between a first position wherein the lid is spaced from the base to a second position wherein the lid is adjacent the channel such that the first substance communicates with the input of the channel.

8. The lid of claim **6** further comprising a second seal releaseably connected to at least one of the second membrane and the body adjacent the second membrane, the second seal moveable between a first position wherein the removable seal overlaps the second membrane and isolates the first substance in the first well and a second position wherein the second seal is spaced from the body.

* * * * *