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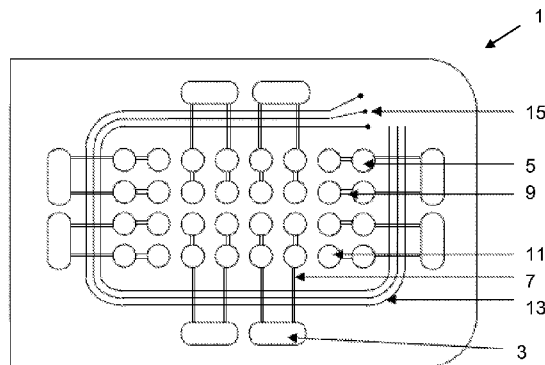


Figure 1

(57) Abstract: A reactor comprising a platform, the platform at least defining: at least one fluid reservoir arranged to receive fluid; a plurality of reaction chambers, wherein the reaction chambers are discrete from each other; a plurality of supply channels for transporting fluid from the at least one fluid reservoir to one or more of the plurality of reaction chambers; pumping means arranged to pump fluid through one or more of a plurality of supply channels. A method of manufacturing the reactor, and the use of the reactor and a kit comprising the reactor.

**REACTOR**

The present invention relates to reactors, such as bioreactors, a method of making a reactor, the use of a reactor and a kit for making or using a  
5 reactor.

As cell based biological research continues to develop at a rapid rate, there has been an increasing interest in bioreactors and methods to make cell culturing and analysis more efficient. Larger scale systems are  
10 required when attempting to acquire a large cell number for clinical uses, however smaller scale systems are desired for research purposes to improve analysis techniques and minimise the number of cells and reagents used.

15 Spinner flasks, perfusion systems, stirred vessels and rotating wall bioreactors have been a major focus of bioreactor development; however the area of microscale bioreactors is a growing field. The ability to study cell-cell and cell-matrix interaction on a small scale reduces the cost of often expensive materials as well as the culturing time associated with  
20 large cell numbers. Channels and culture wells can be designed for specific needs depending on the desired field of research. Biosensors can be inserted to monitor pH levels, glucose levels, electrical fields and so forth.

25 Perfused culture is preferred over static culture as it ensures a consistent flow of nutrients and removal of metabolites as opposed to fluctuations which result from regular media changing. An automated perfusion system also reduces the need for manual involvement.

30 A lab-on-a-chip system is one example of a perfusion based cell culture system. A lab-on-a-chip is a device that integrates one or several

laboratory functions on a single chip of only millimeters to a few square centimeters in size. Lab-on-a-chip systems deal with the handling of extremely small fluid volumes down to less than pico liters. A typical a lab-on-a-chip reactor for continuous cell culture incorporates a  
5 micropump, microchannels and a microculture platform (Wu M-H *et al.* 2008. *Sensors and Actuators B*, 129, 231-240).

Unfortunately there are a number of problems with the lab-on-a-chip design. These include cell loading, analytical, and operational problems.  
10 Performing many standard functions required from a regular static tissue culture system is not possible with a lab-on-a-chip system. Problems include an inability to provide variable surface treatments for monolayer cultures, restrictive collagen/cell hydrogel input techniques, collagen/cell hydrogel breakdown, incompatibility with standard analysis techniques,  
15 and lack of flexibility of the system for use with other cell types. By way of an example, some of the problems of existing lab-on-a-chip designs and methodology are described as follows.

#### **Surface Treatment for Monolayer Cultures**

20 For sufficient cell adhesion in a monolayer culture, the surface of the cell culture platform often needs to be treated differently from the surface of the inlet wells. The inlet wells require treatment with selected surfactants to prevent protein adsorption. However in order to pump fluid through the inlet wells in typical lab-on-a-chip designs, the fluid must pass over  
25 the culture platform thereby also exposing this section of the bioreactor. Thus, it is difficult to treat the inlet wells and the microculture platform differently.

#### **Collagen/cell input and breakdown**

30 For lab-on-a-chip designs cells are often provided in a collagen hydrogel, which allows the cells to be easily inserted into the system and to grow in

a 3D environment. However, inputting the collagen/cell hydrogel into the chip can lead to problems. Collagen is liquid when acidic and when it is neutralized it forms a gel, however to avoid gelling too early after neutralization it needs to be kept on ice. When introducing the collagen/cell hydrogel into a typical lab-on-a-chip design, a microvalve needs to be connected to a compressed air supply and inflated to ensure that the collagen/cell hydrogel does not leak from the culture platform into the microchannels. This requires connection to an air supply until the collagen has completely gelled (approximately 30 minutes after transfer to a 37°C atmosphere). This is usually done in a desktop incubator because traditional incubators do not provide a compressed air supply. As a consequence, it is difficult to maintain sterility when connecting the bioreactor to the compressed air supply and the collagen/cell hydrogel input while it is on ice, and then gelling the collagen at 37°C.

An additional problem with the collagen/cell hydrogel input is that if the collagen/cell hydrogel is drawn too quickly through the cell culture platform, undesirable air bubbles can form in the reaction wells, and if the collagen/cell hydrogel is drawn too slowly, the collagen will gel before entering the bioreactor making it unable to pass through the inlet port.

When the culture medium is passed through the microchannels to the collagen/cell hydrogel, the medium moves through the pores of the collagen/cell hydrogel and tends to leak from the collagen/cell hydrogel inlet and outlet ports. Although attempts may be made to cover these ports following collagen/cell hydrogel input, it is difficult to form a sterile, complete, seal.

Also, the rate at which culture medium needs to be pumped through the system to feed the cells causes the collagen/cell hydrogel to degrade leaving little or no cells in the wells.

## 5 Analysis

Multiphoton or confocal microscopy can be used to view cells undergoing advanced optical functional assays in lab-on-a-chip bioreactors. However, viewing the cells through the relatively thick walls of such bioreactors can lead to distorted images and optical breakdown. Thus, current  
10 bioreactor design does not allow for accurate studies of the cells as the intracellular features and detailed morphological characteristics cannot be identified.

### Alternative Cell Types

15 An additional disadvantage of current lab-on-a-chip bioreactors is that they are not flexible for use with all/most cell types, for example, they cannot be readily used with embryonic stem cells. Embryonic stem (ES) cells may need to be first grown on a layer of feeder cells and it would be difficult to line current lab-on-a-chip bioreactors with such feeder cells.  
20 The lab-on-a-chip bioreactors often offer large surface to volume ratio, which may affect cell functions. This is particularly important to stem cells.

Due to the small dimensions of lab-on-a-chip systems they do not easily  
25 integrate with standard laboratory equipment. They are also limited by the relatively small cell culture sizes, which can limit the analysis that can be performed.

It is an object of the present invention to provide a reactor which  
30 overcomes at least some of the above problems.

According to a first aspect of the invention, there is provided a reactor comprising a platform, the platform defining any one or more, and in some embodiments all, of the following:

- at least one fluid reservoir arranged to receive fluid;
- 5     • a plurality of reaction chambers, wherein the reaction chambers are discrete from each other;
- a plurality of supply channels for transporting fluid from the at least one fluid reservoir to one or more of the plurality of reaction chambers;
- 10    • pumping means arranged to pump fluid through one or more of a plurality of supply channels.

The reactor may be a bioreactor (for example, suitable for use with organisms).

15

The reactor advantageously enables cellular analysis with a reduced cell number and growth in an automated fashion. The reactor is advantageously capable of handling many cell types including those in suspension, surface-adherent cells, and cells in 3D culture. The reactor  
20 provides the benefit of easier handling because fewer external tube connections are required relative to conventional bioreactors. In particular, an external fluid supply, for example, for media, is unnecessary because a reservoir of fluid can be held in the platform.

- 25    In one embodiment, the only external connection may be an air/gas supply needed for the pumping means.

The platform may further define at least one outlet channel extending from at least one of the reaction chambers, preferably from each of the  
30 reaction chambers, wherein the outlet channel is suitable for transporting fluid away from the reaction chamber.

The platform may further define at least one collection reservoir arranged to collect fluid flowing from at least one outlet channel. Preferably the platform defines a plurality of collection reservoirs. The platform may  
5 define at least one collection reservoir per reaction chamber.

The collection reservoirs provide a benefit that they reduce the need for additional tubing from the platform. Reducing the amount of tubing required can advantageously reduce the need for manual handling and the  
10 possibility of contamination.

The platform may have a top-surface and may comprise a base. The top-surface may be defined as the top most surface of the platform when in use. The platform may comprise a first layer, a second layer, and a third  
15 layer. A surface of the first layer may comprise the top-surface. The third layer may comprise the base. The second layer may be sandwiched between the first and third layers.

Where reference is made to layers, the layers may be actual separate  
20 layers of material joined together in the manufacture of the platform, or the layers referred to may be physical portions of the platform, not determined by the construction of the platform.

The reaction chambers and/or fluid reservoir and/or collection reservoirs  
25 may be defined at least partly by the first layer, and may be formed within the first layer. The outlet channel(s) may be defined at least partly by the first layer and may be formed within the first layer, preferably the outlet channel is formed in the top-surface.

30 A benefit of the reaction chambers and/or fluid reservoir and/or collection reservoirs being defined by the first layer is that they are easily

accessible from the top of the platform, with no other channels in the way.

A benefit of the outlet channel(s) being defined in the first layer, and preferably in the top-surface of the platform is that it can act as an overflow channel for fluid accumulating in the reaction chamber(s) without the need for additional pumping, and preventing any undesirable pressure increases. Providing the outlet channel in the top-surface of the platform also ensures that the contents of the reaction chamber are not swept away or disintegrated, for example, cells and/or scaffolding will remain near the bottom of the reaction chamber due to gravity and they will not be undesirably swept into the outlet channel.

The second layer may have the supply channel(s) formed in it, and may define, at least partly, the supply channel(s) and the third layer may define, at least partly, the pumping means.

A benefit of the supply channel(s) being provided in the second or third layers is that they can supply fluid into the bottom of the reaction chambers, which ensures good mixing of the fluid when entering the reaction chamber.

The supply channel(s) and the outlet channel(s) may not be on the same plane, i.e. they may be vertically spaced in use. The supply channel(s) and the outlet channel(s) may be defined in different layers. Preferably the outlet channel(s) is arranged in use to be above the supply channel(s).

An advantage of providing vertically spaced supply channel(s) and outlet channel(s) is that fluid can be supplied by the supply channel(s) into the bottom of the reaction chamber and excess fluid or waste can overflow from the reaction chamber through the outlet channel(s) near the top of

the reaction chamber. This ensures that fresh feed reaches the cells while the spent media (which can be toxic) is removed. The fresh media enters at the bottom of the wells, where the cells are, and the spent media is pushed upward and away from the cells and may be discarded via the  
5 overflow channel.

The reaction chambers may be in the form of discrete wells, such as the wells of a conventional 96-well plate. A single reaction chamber may have a volume of between 10 and 2500  $\mu\text{l}$ , or between 50 and 1000  $\mu\text{l}$ ,  
10 ideally 50-500  $\mu\text{l}$ .

The size of the reaction chambers advantageously provides less surface to volume ratio than smaller conventional lab-on-a-chip type reactors. Too much surface to volume can have the effect of interfering with the  
15 reaction, for example, cells may disadvantageously adhere to the surface. The size of the reaction chambers can also advantageously provide enough space for 3-D structures in the reaction chambers, for example, for the provision of scaffolding structures in the reaction chamber.

20 Where reference is made to the reaction chambers being discrete from each other it is intended that the reaction chambers may be separate from each other, for example separate wells, with no directly connecting channel or aperture there between, and therefore not in fluid communication. In particular, the reaction chambers are independent of  
25 each other.

The platform may be arranged such that, in use, liquid does not flow, or perfuse from one reaction chamber to another reaction chamber.

30 Having discrete/separate reaction chambers avoids cross-contamination of adjacent reaction chambers, which provides the benefit of more accurate

results. Different cell types or cell seeding densities can be provided for different reaction chambers. Advantageously, different conditions or experiments can be run in parallel on the same plate. An additional benefit is that different reagents can be provided to the discrete/separate  
5 reaction chambers without cross-contaminating adjacent reaction chambers.

One or more of the reaction chambers may be connected to a further reaction chamber by a connection channel that allows diffusion or flow of  
10 molecules between each of the one or more reaction chambers and the respective further chamber to which it is connected. The reaction chambers in a connected group of reaction chambers may be connected in parallel, such that each reaction chamber has a supply channel and an outlet channel. Alternatively, one or more reaction chambers, for example  
15 two or three reaction chambers, may be connected in series, such that they are supplied by a single supply channel, whilst there are other discrete reaction chambers or groups of chambers on the reactor.

Two or more connected reaction chambers may be connected to each  
20 other but discrete/separate from one or more other reaction chambers.

Each reaction chamber may be individually accessible, for example, for addition or removal of contents, reagents, or cells from the reaction chamber. Preferably the reaction chambers are individually accessible  
25 from the top-surface of the platform. The reaction chambers may be open-wells formed in the top-surface of the platform.

The provision of individually accessible reaction chambers, which are open-wells and accessible from the top-surface of the platform, provides  
30 the benefit that the content of the reaction chambers can be changed, removed, or added-to, without disturbing other reaction chambers, and/or

without destroying the bioreactor. Such a bioreactor is advantageous over traditional lab-on-a-chip bioreactors where cells are trapped within the bioreactor, thus, many standard analytical techniques cannot be performed such as RT-PCR, immuno-localisation, and FACS (Fluorescence  
5 Activated Cell Sorting), without destroying the bioreactor in order to remove the cells.

The open-well reaction chambers also provide a benefit that microscopy can be used to study cell morphology without having to focus through a  
10 thick wall of PDMS, unlike current bioreactors. The open-well reaction chambers also provide a benefit that problems with trapped bubbles or pressure problems in the reaction chamber are minimized, if not eliminated.

15 Preferably the reaction chambers are capable of retaining a cell culture. The cell culture may be a cell suspension in a liquid. The cell culture may be a cell suspension in a solidified gel, or partially solidified gel. The cell culture may be seeded in, and/or on, a scaffold. The cell culture may be adhered to the reaction chamber walls and base.

20

The reaction chambers have the benefit that most cell types can be cultured, retained, maintained and grown therein.

The reaction chambers may be arranged to receive fluid and/or solid  
25 material, such as a scaffold.

The fluid may be a liquid. The fluid may be cell culture media. The fluid may comprise any of the group selected from peptides, such as antibodies, prions, enzymes, proteins, or signalling peptides; cells, such  
30 as cells from the monera, animalia, plantae, fungi, and protista kingdoms; nutrients; agents/drugs, such as antibiotics, test compounds, inhibitors, or

hormones; viruses, for example mammalian viruses or phage; and nucleic acids, or combinations thereof.

The platform may comprise, or be formed of, a transparent material. The platform may comprise a flexible material, preferably a flexible polymer. At least one of the first, second, and third layer of the platform may comprise, or be formed of, a flexible polymer. The platform may comprise, or be formed of, an air permeable material, preferably an O<sub>2</sub> and/or CO<sub>2</sub> permeable material.

10

The platform may comprise, or be formed of, a polymer, preferably a gas permeable polymer, such as polydimethylsiloxane (PDMS), polypropylmethylsiloxane (PPMS), polytrifluoropropylmethylsiloxane (PTFPMS), or polyphenylmethylsiloxane (PPHMS).

15

Such polymers have an advantage that they can be easily cast into a specific shape, whether it is macroscale or microscale (Microscale is intended to refer to lab-on-a-chip technology (usually sub-millimeter range). Macroscale involves any larger structures). The polymers, such as PDMS, are beneficial for use in biological processes due to its biocompatibility. They can be chemically inert, nontoxic, inexpensive and optically transparent (allowing for easy cell-monitoring through the material). The polymers also have an advantage of being gas permeable, allowing for exchange of oxygen and carbon dioxide between the cells and their external environment (Lounaci M *et al.* 2007 *Microelectronic Engineering*, 84, 1758-1761). An added benefit of the polymers is that they are capable of being bound to other polymer layers either reversibly or irreversibly.

In an embodiment where the platform comprises, or is formed of polymer, such as PDMS, the reaction chambers may comprise, or be

formed of, a different material, such as standard tissue culture plastic or glass.

In an embodiment where the platform comprises, or is formed of  
5 polymer, such as PDMS, the polymer may be coated with poly-L-lysine or other agents in order to encourage cell growth.

The platform may be sized substantially the same as an industry/research standard microplate plate, for example a 96-well microtitre™ plate. The  
10 platform may be between about 3 cm and about 300 cm in length and between about 3 cm and about 300 cm in width. The platform may be about 128 mm in length and about 85 mm in width, preferably about 127.76 mm  $\pm$  0.25 mm in length and about 85.48 mm  $\pm$  0.25 mm in width.

15 Providing a platform with the same dimensions as a standard 96-well microplate advantageously allows for the cells/reagents within the bioreactor to be analysed using standard industry/laboratory equipment. For example, the whole bioreactor can be placed into a microplate reader  
20 for analysis with research tools such as alamarBlue™. Additionally, enough space will be provided such that a microscope objective could be positioned close enough to the platform without interfering with any inlet/outlet tubes. The size of the platform advantageously allows multiple reactions to be conducted whilst still providing room for adequately large  
25 sized reaction chambers.

The pumping means may be arranged to act directly upon the supply channels. The pumping means may act upon individual supply channels, or simultaneously on a plurality of supply channels.

Preferably the pumping means is arranged such that, in use, it does not contact fluid in any of the supply channels, fluid reservoir, outlet channel, reaction chambers, or collection reservoir. The pumping means may be physically separated from the supply channels by a wall or  
5 membrane.

Each supply channel may have a separate/individual pumping means. The platform may have a single pumping means arranged to pump fluid through all the supply channels in the platform.

10

The pumping means may be a micropump. The pumping means may be a peristaltic membrane-based pneumatic micropump. The pumping means may pump fluid through the supply channel(s) by deflection of a flexible wall of the supply channel(s), or a membrane in the wall of the supply  
15 channel. The pumping means may be a micropump of the type described by Wu M-H. *et al.* (2007. *Sensors and Actuators B* 129, 231-240); Wu, M-H. *et al.* (2007. *Biomed Microdevices* 10, 309-319) or Unger *et al* (*Science* 2000, Vol. 288 pp113-118).

20 The pumping means may comprise at least one pump channel, wherein the pump channel is arranged to traverse at least one supply channel. The pump channel may be inflatable by air pressure (i.e. air or any suitable gas), and/or liquid pressure, preferably the pump channel is inflatable by air pressure. The pump channel may traverse the at least one supply  
25 channel, at least once, at least twice, or at least three times. Where the pump channel traverses the supply channel at least twice or at least three times, preferably the pump channel traverses the supply channel at different areas along the length of the supply channel. The pump channel may be substantially S-shaped. The pump channel may comprise a series  
30 of U-shaped bends.

The pumping means may comprise, or be formed of, a plurality of pump channels arranged to traverse at least one supply channel. Each pump channel may be arranged to deflect a different wall area of the same supply channel along the length of the supply channel.

5

The pumping means advantageously allows the treatment of the supply channels with agents, such as surfactants, without affecting the reaction chamber. In particular, the pump channels (used for the pump) can be filled with air or liquid to separate the reaction chamber from the supply channels.

10

The platform may further define at least one pump inlet aperture for connecting the at least one pump channel to an external air/gas supply, or external liquid supply. Preferably the platform defines one pump inlet aperture per pump channel. The at least one pump inlet aperture may be defined in the top-surface of the platform.

15

The at least one pump channel may be connected to at least one solenoid valve for controlling the external air/gas supply or liquid supply.

20

The platform may define between 1 and 150 reaction chambers, preferably 16 reaction chambers, for example, in 128x85 mm microtitre plates.

Providing 16 reaction chambers advantageously provides adequate spacing for storage of fluid in the fluid reservoir, the reaction chambers, the collection reservoirs, the supply channels, the outlet channels and the pumping means.

25

The number of fluid reservoirs may be equal to the number of reaction chambers. The number of fluid reservoirs may be half, or a third, of the

30

number of reaction chambers. Preferably the at least one fluid reservoir is arranged to supply fluid to one, two, three or more reaction chambers.

Having a fluid reservoir supplying two or three reaction chambers  
5 advantageously allows experiments on cells to be done in duplicate or triplicate.

The number of collection reservoirs may be equal to the number of reaction chambers.

10

Having an equal number of collection reservoirs to reaction chambers allows each reaction chamber to have a collection reservoir for individual analysis of the reaction/cell culture products from each reaction chamber.

15 The bioreactor may comprise a lid arranged to fit over the platform. The lid may be removable.

The lid has a benefit that it can help to prevent contamination of the contents of fluid or cell culture in the bioreactor. The lid also has a  
20 benefit that it can help to prevent evaporation of fluid from the bioreactor. The lid being removable advantageously allows for the fluid and/or cells to be easily extracted, for example, without destroying the bioreactor. A removable lid also provides an advantage that problems with trapped bubbles or pressure problems are minimized.

25

The platform may comprise means for heating or cooling the platform. The means for heating or cooling the platform may be a thermal plate. Preferably, the thermal plate has high thermal conductivity. Preferably the thermal plate is metal. The thermal plate may be arranged to maintain  
30 the platform at a specified incubation or reaction temperature, for

example at 37°C. The thermal plate may form a base layer for the platform.

5 An advantage of incorporating a means for heating or cooling the platform is that the bioreactor does not have to be used inside an incubator.

10 In one embodiment, two or more reactors may be coupled together. Two or more reactors may be connected in series, or in parallel. The collection reservoir of one reactor may be connected to the fluid reservoir of a second reactor, for example by a channel, or tubing.

15 Connecting two or more reaction chambers has a benefit that experiments or test could be connects in series or parallel. A reactor may condition the media, or provide necessary constituents, to be used in a second reactor connected to the first reactor.

20 According to another aspect of the invention, there is provided a method of manufacturing a reactor, preferably a bioreactor, comprising at least one, and preferably all, the steps of:

- forming a first layer, the first layer defining, at least in part, a plurality of reaction chambers, and at least one fluid reservoir;
- forming a second layer, the second layer defining at least one supply channel;
- 25 • forming a third layer, the third layer defining pumping means;
- assembling the second and third layer together, and assembling the first layer with either the second layer or the third layer to form a platform comprising a first, second and third layer.

30 At least one of the first, second and third layers may comprise, or be formed of, a flexible polymer. The flexible polymer may comprise

polydimethylsiloxane (PDMS), polypropylmethyilsiloxane (PPMS), polytrifluoropropylmethyilsiloxane (PTFPMS), or polyphenylmethyilsiloxane (PPHMS). Preferably all three layers comprise PDMS.

5

The at least one supply channel of the second layer may be provided by soft lithography.

Soft lithography is a set of methods for fabricating or replicating structures using elastomeric stamps, moulds, and conformable photomasks (Rogers JA *et al.* 2005. *Materials Today*, 8, 50-56).

10

Preferably the pumping means defined by the third layer comprises at least one pump channel. The at least one pump channel of the third layer may be provided by soft lithography.

15

The first layer may be formed by moulding.

The first, second and third layers may be clamped and/or bonded together to form the platform.

20

The method may further comprise the step of puncturing at least one hole through the first layer, or the first layer and the second layer, to form at least one pump inlet aperture extending from the pump channel through the first layer, or first layer and second layer, for connection of the pump channel to an external air/gas supply or liquid supply.

25

The first layer may further define at least one outlet channel.

The at least one outlet channel of the first layer may be provided by a mould.

30

The first layer may further define, at least in part, at least one collection reservoir.

- 5 According to another aspect of the invention, there is provided a use of the bioreactor according to the invention for at least one of cell culture and cell analysis.

According to another aspect of the invention, there is provided a kit  
10 comprising the bioreactor according to the present invention and instructions.

It will be appreciated that, where appropriate, all optional or preferable features applicable to one aspect of the invention can be used in any  
15 combination, and in any number. Moreover, they can also be used with any of the other aspects of the invention in any combination and in any number. This includes, but is not limited to, the dependent claims from any claim being used as dependent claims for any other claim in the claims of this application.

20

Embodiments and examples of the present invention will now be described herein, by way of example only, with reference to the following figures.

- 25 **Figure 1** - shows a plan view of a 96-well-sized bioreactor;

**Figure 2** - shows a side-view of the bioreactor with the scaffold inserted and the three layers attached;

- 30 **Figure 3** - shows flow regime of deflected PDMS membranes in the micropump;

**Figure 4** - shows a design of the bioreactor mould. **Figure 4A** - a plan-view of the bioreactor base; **Figure 4B** - a cross-sectional side view of the bioreactor base; **Figure 4C** - a photograph of the mould used to cast  
5 PDMS to make the bioreactor base;

**Figure 5** - is an illustration of the microchannel and micropump layers of the bioreactor system. **Figure 5A** - the microchannel layer; **Figure 5B** - the micropump layer;  
10

**Figure 6** - shows a photomask used in the development of the micropump;

**Figure 7** - shows the structure of the end of one of the microchannels;  
15

**Figure 8** - shows the result from a Tepla Surface Profiler showing the thickness of the SU-8 25 channel at approximately 65  $\mu\text{m}$  and the thickness of the PDMS at approximately 105  $\mu\text{m}$ ;

20 **Figure 9** - shows the structure of polydimethylsiloxane;

**Figure 10** - shows the structure of Pluronic F68 (a) POE (b) POP;

**Figure 11** - illustrates Pluronic F68 adsorption behaviour on PDMS.  
25 **Figure 11A** - densely packed monolayer, optimal concentration; **Figure 11B** - loosely packed monolayer, low concentration; **Figure 11C** - bilayer, high concentration; **Figure 11D** - micelle/aggregate, high concentration;

**Figure 12** – shows a clamp designed to hold the layers tightly together. **Figure 12A** - clamp without the bioreactor; **Figure 12B** - clamp holding the bioreactor;

5 **Figure 13** – shows an electrical & control system for the reactor; and

**Figure 14** – shows the set-up of six bioreactor systems in a desk-top incubator.

10 **Figure 15** – shows an alternative layout of a bioreactor, with two reaction wells connected in series.

## **Development of Perfused Microbioreactors for 3D hMSC Culture**

### **Introduction**

15 A bioreactor was designed for perfused 3D hMSC culture. There were many problems with the previously described perfused lab-on-a-chip bioreactor and so a new bioreactor was designed.

### **Design Concepts**

20 The dimensions of the overall system are the same as that of a 96-well plate to increase the compatibility with standard analysis equipment. To allow adequate spacing for storage of the fresh culture medium, the culture wells, waste collection, the microchannels and the micropump, only 16 wells are incorporated into the plate.

25

Figures 1 and 2 are representative illustrations of the bioreactor (1) with the three PDMS layers attached (17, 19, 21). The reactor (1) has a first thick PDMS layer (well base) (17) which has several outer chambers forming fluid reservoirs (3) defined within it where fresh inlet medium is held. The outer chambers (3) are connected to culture wells/reaction chambers (5), also formed in the first layer, by microchannels forming

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supply channels (7) which are defined in a second thinner PDMS layer (microchannel layer) (19) beneath the first PDMS layer (17). The microchannels (7) lie on the bottom of the system, such that they each extend from an aperture in the bottom of an outer chamber (3) to an aperture in the bottom of a culture well (5).

The outer chambers (3) are placed at specified distances away from the culture wells (5) themselves to allow for enough room for the micropump/pumping means (13) to run between. Each culture well (5) has an overflow channel (9) extending from the top of the culture well (5) to a collection reservoir (waste media chamber) (11) in order to collect spent media from the top of the well (5).

The micropump (13) is defined in a third thin PDMS layer (21) which is beneath the first and second PDMS layers (17, 19). The micropump (13) comprises three separate micropump channels (23) extending parallel to each other and extending around all four sides of the reactor (1) in a substantially rectangular configuration. Each micropump channel (23) has an air inlet (15) and is sealed at the other end. The air inlet (15) can be connected to a controlled air supply. The micropump channels (23) cross perpendicularly beneath the microchannels (7) of the second PDMS layer (19), and are separated from the microchannels (7) of the second PDMS layer (19) by a thin membrane that is capable of deforming into the space of the microchannels (7) by air or liquid pressure applied to the micropump channels (23).

In this embodiment of the reactor (1), each single outer chamber (3) is connected to (supplies media to) two culture wells (5) by respective microchannels (7) each of which is traversed by the micropump (13). However, alternative configurations are possible, such as connection of the outer chamber (3) to one, three, four, or more culture wells (5).

Additionally, in this embodiment, each culture well (5) is discrete from the other culture wells (5), i.e. not connected to the other culture wells downstream of the micropump, such that no molecules or fluid can flow between them. In alternative embodiments, groups of two, three, four or  
5 more culture wells (5) may be in fluid connection with each other by a connecting channel, but not with other groups of culture wells (5) from which they remain discrete and unconnected. Thus, the effects that neighbouring cells/reactions have on each other may be studied.

10 As the wells (5) are connected in parallel to one another, and not in series, this reactor (1) has overcome the problem from the lab-on-a-chip style which allows cross-talk between culture wells. The system is not sealed and has a similar lid to standard tissue culture plates. This ensures that there are no problems with trapped bubbles or pressure problems  
15 and, most importantly, the cells can be easily removed for analysis. This set-up also allows for cells to be grown in adherent, suspension, or 3D cultures. The system can also be incorporated into a multiphoton microscope set-up for real-time imaging with high objective strength. Unlike the previously described reactor, the PDMS from the bottom of  
20 the culture wells (5) can be removed and replaced with a different material such as standard tissue culture plastic or glass coverslips, or just left as PDMS and coated with poly-l-lysine to permit cell growth. Not only does this make the system more compatible with standard culture systems, but it also overcomes the surface treatment problem. The  
25 microchannels (7) can first be treated to prevent protein adsorption and then the bottom of the culture wells can be replaced with a different material.

An alternative layout of a bioreactor, with two reaction chambers (5)  
30 connected in series by a connection channel (6), is shown in Figure 15. Although, two reaction chambers (5) are connected in series, there are

still discrete groups of reaction chambers (5) that are connected in parallel relative to each other and not connected in series. The series connection between two reaction chambers (5) could, for example, provide a first well that pre-conditions media, which then flows through  
5 the connection channel (6) to the second reaction chamber.

A cross-sectional schematic of the micropump (13) is shown in Figure 3. As previously mentioned, the micropump (13) is driven by air. As air enters the micropump (13) it deflects the thin membrane layer (101),  
10 interfering with the culture medium microchannels (7) and causing the medium to flow to the cell culture wells (5) from the outer chamber/reservoir (3). The pattern of membrane deflection between the three channels (23) in the micropump (13) is shown in Figure 3. In step one, the channel (7) is open. Step two, the first membrane (101) comes  
15 down pushing half of the membrane volume of the medium to the right and half to the left. Step three has a second membrane (101) deflect, pushing medium equal to the full volume of the membrane in the direction of cell culture well (5). In step four, a third membrane (101) deflects pushing another membrane volume in the direction of the cell culture well  
20 (5). Steps 5 and 6 involve the first and second membranes (101) retracting to refill the microchannel (7). The system is then restored to step 1 where the third membrane (101) has retracted and culture medium flows in from both sides. Going through a complete cycle, the net volume of media travelling to the cells is equivalent to two membrane volumes.

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### **Fabrication & Assembly**

The 96-well sized bioreactor incorporates macro- and micro-sized components. The macro-sized base has been developed using a machined mold and the micro-sized channels and pumps are created using soft-  
30 lithography.

**Base**

A drawing of the base layer (17) of the bioreactor is shown in Figure 4. This includes a plan-view (Figure 4A) as well as a side profile of the channels (Figure 4B).

5

The mould shown in Figure 4C was used for the casting of the base layer (17) of the bioreactor. A 10:1 ratio of PDMS (Sylgard 184, Dow Corning) monomer and crosslinking agent was used to make the PDMS and poured into the mould. The PDMS was left a room temperature for 1  
10 hour to allow the bubbles to burst, and then heated at 75oC for 4 hours.

**Construction of the micropump and microchannel layers****Soft Lithography**

Soft lithography was used to construct the micropump and microchannel  
15 layers (19, 21). Soft lithography is a set of methods for fabricating or replicating structures using elastomeric stamps, moulds, and conformable photomasks (Rogers JA *et al.* 2005. *Materials Today*, 8, 50-56).

Soft lithography was used to develop a mould, or template, which can be  
20 used to accurately and repeatedly develop the microstructures of the bioreactors including the microchannels (7) and the micropumps (13). This involves first drawing the desired patterns, representing the channels (7) or the pump (13), using a standard computer aided design programme. Figures 5A and 5B show the initial drawings for the microchannel and  
25 micropump layers (19, 21) respectively. Following this, the drawings are sent for printing to develop a photomask as shown in Figure 6. The photomask is a transparent blank which is coated with a chrome metal adsorbing film in all regions except for those of the desired patterns as shown in Figure 6.

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Once the photomask has been developed, the silicon wafer needs to be prepared. A silicon wafer is coated with SU-8 photoresist. SU-8 is a negative, epoxy-type, near-UV photoresist (365 nm) consisting of eight epoxy groups.

5

Two four inch silicon wafers were cleaned with acetone, rinsed with deionised water and dried with a nitrogen gun. The wafers were further dried by heating on a hot plate at 200°C for five minutes and then left to cool. SU-8 25 (Microchem, MA, USA), which had been left at room  
10 temperature for a minimum of two hours, was used to coat the silicon wafers. After optimization of the spinning speeds for obtaining particular thicknesses, the SU-8 25 was spun onto the wafers.

The wafer to be used for the micropump needed an SU-8 thickness of 25  
15  $\mu\text{m}$  and so the sample was spun at 2000 rpm by accelerating at 100 rpm/sec until 500 rpm and then at 300 rpm/sec until 2000 rpm and left to spin for 30 seconds. Spinners are commercially available and incorporate a vacuum suction to hold the wafer in place and allow for spinning at a desired speed.

20

The microchannel layer (19) required an SU-8 thickness of 65  $\mu\text{m}$  and so this wafer was spun at 1000 rpm by accelerating at 100 rpm/sec until 500 rpm and then at 300 rpm/sec until 1000 rpm and left to spin for 30  
25 seconds. The wafers were pre-baked at 65°C for 10 minutes with a heating rate of 10°C every 5 minutes. The solvent in the SU-8 must be removed before it is exposed to UV treatment and so it is soft-baked at 95°C for an optimised time depending on the thickness. This is then cooled slowly to avoid thermal shock. Following the solvent removal the wafer is ready for UV exposure. In this case soft baking was done at 95°C for 70 minutes at  
30 the same heating rate. The cooling rate was -10°C every five minutes until room temperature.

The wafer coated with 25  $\mu\text{m}$  of SU-8 was then inserted into a Karl Suss Mask Aligner 6 (SUSS Micro Tech), aligned with the micropump photomask, and was exposed using a UV lamp at an intensity of 10 mW/cm<sup>2</sup> and exposure wavelength of 365 nm for a total exposure dose of 100 mJ/cm<sup>2</sup>. The exposure time is again dependent on the thickness of the SU-8 layer and will lead to cracks in channels or channels not fully developing if the UV exposure time is too high or too low respectively.

Subsequently, this wafer was removed and the thicker-coated wafer was then placed into the Mask Aligner, along with the microchannel photomask, and the same lamp intensity and wavelength was used, but with a total exposure dose of 150 mJ/cm<sup>2</sup>. The wafers were then post-exposure baked at 65°C for one minute and 95°C for three minutes, at a heating rate of 10°C every five minutes and a cooling rate of -10°C every five minutes in which the SU-8 polymerises through a cationic photo amplification mechanism. That is, the polymerisation is catalysed by a Lewis acid which is generated through the UV illumination. The Lewis acid breaks the epoxy bond therefore opening up the ring structure and allowing the molecules to form polymer chains. Only the clear sections of the photomask, with the desired patterns, are exposed to the UV treatment and so these are the only sections which polymerise.

The wafers were finally rinsed in EC Solvent with agitation for 6 minutes, then rinsed with isopropanol and dried with a nitrogen gun. This removes any SU-8 not exposed to the UV treatment, and leaves the desired microstructures.

The height of the SU-8 structures was measured using the TepLa Surface Profiler. A microscope was used to view that the structures had clearly formed as shown in Figure 7.

When needed for PDMS casting, the wafers were rinsed with acetone and dried with nitrogen. Sylgard 184 and its corresponding crosslinker were thoroughly mixed in a ratio of 10:1 respectively, and poured onto the wafers. The micropump wafer was first placed in a disposable paper tray to prevent leaking of the PDMS and covered to a depth of approximately three mm. The wafer with the microchannels was placed in a spinner, coated with PDMS, and spun at a speed of 800 rpm, with an acceleration of 100 rpm/second, left for 30 seconds at 800 rpm, and then decelerated at a rate of 100 rpm/second. This was left on a hot plate at 50°C until all of the bubbles burst and then left at 65°C for approximately three hours. The Tepla Surface Profile was again used to measure the thickness of the PDMS coated on the wafer as shown in Figure 8.

A silicon polymer, PDMS, which is described in more detail below, can then be poured onto this, and once cured, peeled off to leave a PDMS layer with negative imprints of the SU-8 patterns. This can either be a thick layer of PDMS, or to obtain a thin layer, PDMS is poured on top, spun at an optimized speed to obtain the desired thickness, and heated until polymerised. These layers can then be stuck together reversibly or irreversibly as described below.

### **Polydimethylsiloxane (PDMS)**

PDMS is prepared by thoroughly mixing a polysiloxane base, tetra(trimethylsiloxy)silane, and a curing agent, tetramethyltetravinyldyclotetrasiloxane, in a ratio of 10:1 by weight to form the cross-linked PDMS as shown in Figure 9. After curing, the layers can be reversibly or irreversibly bound together. The outer surfaces of the PDMS are lined with methyl groups causing a hydrophobic surface (Xiu Y *et al.* 2006. *IEEE CPMT International Symposium and Exhibition on Advanced Packaging Materials: Processes, Properties and*

*Interfaces*, 11, 98-103). Hydrophobic surfaces have a tendency to adsorb proteins. In defining whether a protein solution will be adsorbed, numerous factors have to be considered including bound ions, surface charge, surface roughness, surface elemental composition, surface energies, and so forth. When the hydrophobic protein adsorbs onto the hydrophobic surface, bound water is released resulting in a large positive entropy change which causes binding (Israelachvili JN 1992. *Intermolecular Surface Forces*).

## 10 Surface Modification

The surface can be modified to become hydrophilic and prevent protein adsorption. Two methods of surface modification were investigated: introduction of micelles or alternation by covalent bonding. Similarly, the surface can be altered to improve cell adherence.

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## Protein Adsorption

Poloxamer 188 (P188), or Pluronic F68 (by BASF Corporation), has shown to be successful in the PDMS surface modification (Boxshall K et. al, 2006. *Surface and Interface Analysis*, 38(4), 198-201). Pluronic F68 is a block co-polymer poloxamer composed of polyoxyethylene (POE) and polyoxypropylene (POP) as shown in Figure 10. The Pluronic series consists of a variety of ratios of POE:POP, but the optimal has been found to be Pluronic F68 (Boxshall K et. al, 2006. *Surface and Interface Analysis*, 38(4), 198-201). POE is hydrophilic whereas POP is hydrophobic and so at the correct concentration, the POP can be used as a base to bind to the hydrophobic surface allowing the hydrophilic POE ends to protrude and provide a hydrophilic layer which repels the proteins. This is illustrated in Figure 11 and shows the behaviour of the Pluronic F68 when the surface is treated with different concentrations.

30

Figure 11A is the optimal regime of polyoxyethylene (161) and polyoxypropylene (163). At a lower concentration, as shown in Figure 11B, there is a reduced amount of hydrophilic ends allowing protein adsorption to still occur. At a higher concentration a bilayer can form, as shown in Figure 11C, or micelles/aggregates, Figure 11D, which can lead to uneven distribution of the surfactant and can be washed away easily (Boxshall K et. al, 2006. *Surface and Interface Analysis*, 38(4), 198-201). The optimal condition, as determined by Boxshall et al. (2006. *Surface and Interface Analysis*, 38(4), 198-201), is 3% Pluronic F68 for 24 hours.

10

### Cell Adherence

Pluronic F68 is effective in preventing protein adsorption, but it also prevents cell adherence. To overcome this, various other surface modifiers such as poly-L-lysine can be used or the system can be rinsed with NaOH. Alternatively, the bottom of the wells can be cut out of the PDMS and replaced by glass or plastic slides.

15

### Reversible and Irreversible Binding

An added benefit of PDMS is that it is capable of being bound to other PDMS layers either reversibly or irreversibly. Prior to surface treatment, if both pieces of PDMS are clean and flat, they can simply be stuck together and form a seal as long as no air bubbles are present. To separate these layers, they can be manually peeled apart. To irreversibly bind the layers, plasma oxidation is needed. Prior to surface treatment, the PDMS can be exposed to plasma oxidation to induce an oxidized surface. This enables covalent bonds to be formed between layers. When the PDMS slabs are exposed to plasma oxidation the lower molecular weight PDMS species from within move to the surface causing hydroxyl groups to form and therefore lead to hydrophobic recovery. That is, the plasma oxidation leads to crosslinking and the formation of an inorganic silica-layer consisting partly of silicon bond to three or four oxygen atoms

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25

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(Hillborg H et al., 2000. *Polymer* 41, 6851-6863). When the layers are stuck together, they form covalent bonds. The formation of this silica layer is only temporary and so adhesion should be performed immediately following plasma oxidation. For this reason, plasma oxidation cannot be used to form a permanent hydrophilic surface and so the Pluronic F68 is still necessary. It was thought that perhaps permanent surface modification could be achieved using a combination of plasma oxidation followed by immediate treatment with a solution which could bind to the hydroxyl groups and create a permanent hydrophilic layer. This, however, would not be possible as the microchannels used to transport the culture medium are embedded within thicker layers of PDMS. Hydrophobic recovery as a result of plasma oxidation is known to decrease with increasing thickness of the PDMS and so the oxidation treatment would not be as effective within the structure as it would be at the surface (Lawton et al., 2005 *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 253(1-3), 213-215).

#### **Attachment of the Microchannel Layer to the Base Layer**

Once the PDMS had been cast and set in the three layers, the base (17), the micropump (21) and the microchannel (19), the base PDMS (17) was removed from the base layer mould with care taken to minimize contact with the bottom of the structure. Small holes were cut at the bottom of the PDMS in the inlet wells and the culture wells to correspond to the inlet and outlet of the microchannels (7). Any debris that may have come in contact with the underside of the PDMS was carefully scrapped off and the structure was then plasma oxidized at 1500 ml/min and 100 W for 50 seconds, as determined following optimisation. Immediately following this, the microchannels were cut off the wafer individually using a scalpel and tweezers and aligned with the holes just cut on the larger base structure. Fresh PDMS was then poured on top of the microchannels and the whole structure spun at 800 rpm for 30 seconds with an acceleration

speed of 100 rpm/second and a deceleration speed of 100 rpm/second. This was left at room temperature of two hours and then transferred to an oven at 70°C for three hours to polymerise. Another layer of PDMS was poured on top and this was spun at 600 rpm for 30 seconds with an  
5 acceleration speed of 100 rpm/second and a deceleration speed of 100 rpm/second. This was again left at room temperature of two hours and then at 70°C for three hours. This was then measured with the Tepla Surface Profiler to ensure that the layer was completely flat and to measure the total thickness of the coated PDMS.

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### **Micropump Attachment**

Following the soft lithography process outlined above, the PDMS micropump (21) was removed from the wafer and the necessary section cut out. Holes were punched at the air inlet ports (15) with a needle and  
15 then layers were aligned, stuck together, and clamped to ensure a tight seal. The compiled result is shown in Figure 2.

If there are any air bubbles in the system or the PDMS base is not completely flat, the micropump will not function properly. To overcome  
20 this possibility, clamps were specially designed to hold the layers (17, 19, 21) together tightly and evening and still allow for viewing through the clamp to ensure the micropump (13) is operational (Figure 12).

Metal tubing was inserted into the inlet ports (15) of the micropump (13)  
25 and glued together to ensure that there is a tight seal with no room for air.

### **Electrical Components**

To enable the micropump to operate properly, tubing was connected  
30 between the micropump inlet ports (15) and a series of three solenoid valves (31), with complementing inlet and outlet manifolds, of which the

inlet manifold was connected to the air supply (Figure 13). The solenoid valves (31) were programmed using MatLab to open and close in a pattern which matched the desired pattern of the pumping mechanism shown in Figure 3. That is, it followed the pattern shown in Table 1 where 0 indicates a closed valve and 1 indicates an open valve.

*Table 1: Solenoid valve regime to control the micropump*

| Solenoid 1 | Solenoid 2 | Solenoid 3 |
|------------|------------|------------|
| 1          | 0          | 0          |
| 1          | 1          | 0          |
| 1          | 1          | 1          |
| 0          | 1          | 1          |
| 0          | 0          | 1          |
| 0          | 0          | 0          |

10

The frequency of this cycle is controlled by thumb wheel switches (33) and was initially programmed, using a programmed circuit board (37), to operate in the range of 0.01 Hz to 9.99 Hz in steps of 0.01 Hz. As the programme required that the frequency be determined by dividing by the period, there were rounding errors with the largest error of 0.06 Hz at 9.69 Hz. As the resolution was to operate at 0.01 Hz, this was not an acceptable error. The programme was redesigned to allow the thumb wheel switches (33) to control the period directly, thereby removing the rounding errors completely. The period can be set from 0.1 seconds to 99.9 seconds.

20

Figure 14 shows the electrical controller box (35) responsible for controlling the valves. The electrical components have been designed to allow up to six bioreactors (1) to run simultaneously. There are therefore six separate thumbwheels (33) and air-ports to cater to this specification, with a single air supply (39).

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The set-up for the whole system is shown in Figure 14. The six parallel bioreactors (1) can be operated at different frequencies, enabling the user to conduct parallel experiments with differing flowrates.

5

### **Operating Conditions**

The necessary minimum air pressure and frequency to operate the pump is based on the properties of the PDMS, the culture medium and the dimensions of the bioreactor.

10

First, the minimum pressure to deflect the PDMS membrane is determined. The total minimum pressure required must also take into consideration the hydrostatic pressure from the culture medium in the culture medium well and the cell culture well. Once this is determined, the volume of liquid that is passed through the channels with each deflection cycle is calculated. This is then compared with the desired flow rate to determine the necessary frequency of cycles and consequently the period. The possibility of fluidic resistance is also calculated to determine if it has an affect on the system. Additionally, in some microfluidic systems if the frequency is too high to allow for the micropump channels to completely fill with air, there is a time delay which can lead cause the flowrates to decrease with higher frequencies (Huang et al., 2006. *Journal of Micromechanics and Microengineering*, 16, 2265-2272).

### **Required Pressure for Membrane Deflection**

The necessary air pressure can be determined based on the thickness of the membrane layer and the distance at which it must be deflected. The displacement ( $\delta$ ) of a square PDMS membrane can be determined by equation 6.1 (He et al., 2004. *Journal of Solids and Structures* 41(3,4), 847-857):

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$$\delta = \frac{3 \cdot (2 + \Lambda) \cdot R_e^2 \cdot p}{16\pi \cdot \mu \cdot (1 + \Lambda) \cdot h^3} \quad , \quad \Lambda = \frac{\lambda}{\mu} \quad (\text{Eqn 1})$$

where  $p$ ,  $R_e$  and  $h$  are the pressure differences applied across the membrane, the equivalent radius of the membrane, and the thickness of the membrane, respectively, and  $\lambda$  and  $\mu$  are the Lamé constants. The variable desired is  $p$ , the pressure that needs to be applied across the membrane.

The PDMS must be deflected to a minimum distance of the height of the microchannel. From the microchannel thickness, the required deflection is  $\delta = 65\mu\text{m}$ . The thickness of the PDMS membrane is  $h = 100\mu\text{m}$ .

To determine  $R$ , the equivalent radius, the equivalent diameter is first solved for using the equivalency equation developed by Huebscher (Huebscher, 1948 Friction Equivalents for Round, Square, and Rectangular Ducts. ASHVE Transactions (renamed ASHRAE Transactions)).

$$d_e = 1.30 \times ((a \times b)^{0.625}) / (a + b)^{0.25} \quad (\text{Eqn 2})$$

where,

$d_e$  = equivalent diameter (mm)

$a$  = length of major side (mm)

$b$  = length of minor side (mm)

Solving for equation 2, with  $a$  and  $b$  both equal to 0.25mm and  $d_e$  is 0.273mm, an equivalent radius of  $R_e = 0.137\text{mm}$  is obtained from  $d_e/2 = 0.137\text{mm}$ .

It has been shown that the frequency of PDMS deflection does not affect the shear modulus; this is only affected by temperature (Lötters JC et al.,

1997. *Microsystem Technologies*, 3(2), 64-67). As the system will always be operating at 37°C, it is at this value that all physical properties of PDMS have been acquired for. The shear modulus,  $\mu$ , has been experimentally determined by Lötters et al., (1997. *Microsystem*  
 5 *Technologies* 3(2), 64-67) and found to be approximately 275 kPa at 37°C. Lamé's first parameter,  $\lambda$ , can be found from this, using a common equation for determining elastic moduli for homogenous isotropic materials,

$$\mu = \lambda \frac{1-2\nu}{2\nu} \quad (\text{Eqn 3})$$

10 where  $\nu$ , the Poisson ratio, is 0.4 (Yang FB, 2006. *Thin Solid Films* 515(4), 2274-2283), leaving  $\lambda = 1100$  kPa. A summary of the values needed to solve equation 1 are given in Table 2.

Table 2: Design parameters of the micropump

| Variable  | Value    |
|-----------|----------|
| $\delta$  | 0.065 mm |
| $R_e$     | 0.137 mm |
| $h$       | 0.1 mm   |
| $p$       | Unknown  |
| $\mu$     | 275 kPa  |
| $\lambda$ | 1100 kPa |
| $\Delta$  | 4        |

15

Solving for  $p$  in equation 1, therefore gives, 13.29kPa or 1.93psi gauge. This is therefore the minimum required pressure to deflect the 100 $\mu$ m thick PDMS membrane. As the microchannel is on the bottom of the

bioreactor system, there will be an additional hydrostatic force that needs to be overcome. The hydrostatic pressure is determined by,

$$P = \rho \times g \times h \quad (\text{Eqn 4})$$

where  $\rho$  is the density, 994 kg/m<sup>3</sup> at 37°C,  $g$  is the gravitational acceleration, 9.8 m/s<sup>2</sup>, and  $h$  is the height of the liquid. The maximum height of the liquid when the culture chamber is full is 0.008 m. Solving for  $P$  generates 77.93 Pa, or 0.0113 psi, as the minimum pressure required to deflect the membrane and to overcome the hydrostatic pressure. This increases the required pressure minimally to 1.94 psi.

10

### Frequency Determination

The frequency of the inputted air also needs to be determined. From Figure 3, it can be seen that two deflected membrane volumes of culture media move towards the cells in each cycle. The intersection of the airflow path and the microchannel has dimensions of 0.25 mm by 0.25 mm, or an equivalent radius, from equation (2) above, of 0.137mm. Assuming that the air deflects the membrane to form half a sphere, the volume of the half sphere is 0.00539 mm<sup>3</sup> (from the standard equation of a sphere,  $V = \frac{4}{3}\pi r^3$ , divided by two). As two volumes of deflected membrane get passed through in each cycle, the total volume is 0.0108 mm<sup>3</sup> or 0.0108 µl/cycle.

20

In static culture, hMSC culture medium is traditionally changed once every two days, and for a 96-well plate, the volume in each well is 200 µl. For direct comparison with standard culturing techniques, the desired flow rate is 200 µl/two days, or 0.001157 µl/sec. From the volume flow rate of 0.0108 µl/cycle from above, and this 0.001157 µl/sec, it is determined that the cycle frequency be 0.145 cycles/sec or a period of 6.88 sec. As previously mentioned, the electronic controller is

25

programmed for input of the period. Although 6.88 sec is the desired period to correspond to the standard culturing protocols, the flow rates can be adjusted.

## 5 Air Pressure Time Delay

The two other factors which may affect the flow rate are fluid resistance and a time delay in the air as it moves through the micropump channel. Although it is expected that an increase in frequency would cause an increase in flow rate, if the frequency is too high, there will not be enough time for the micropump channels to fill with air and so there will be a time delay which will lead to a decrease in flow rate (Huang et al., 2006. *Journal of Micromechanics and Microengineering* 16, 2265-2272). To ensure that this is not a problem, the length of time to fill the micropump with air was calculated and compared to the frequency rate.

15

Equation 5 can be used to determine G, the mass flow rate, as this equation describes a system with a pressure change due to compressible air flow when the temperature is kept constant.

$$p_1^2 - p_2^2 = Z_m RT \left( \frac{G}{A} \right)^2 \left( \lambda * \frac{L}{D} + \Sigma \xi + 2 \ln \frac{p_1}{p_2} \right) \quad (\text{Eqn 5})$$

20 Where p1 is the pressure at the start of the pipe, p2 the pressure at the end of the pipe, Zm, the average compressibility coefficient, R, the gas constant of air, T, the air temperature, G, the mass flowrate, A, the cross-sectional area,  $\lambda *$  the friction coefficient, L, the pipe length, D, the pipe diameter, and  $\Sigma \xi$ , the sum of minor losses coefficient.

25

The average compressibility coefficient is determined using,

$$Z_m = \frac{1}{2} (Z_1 + Z_2) \quad (\text{Eqn 6})$$

where Z1 is the compressibility coefficient at the start of the pipe and Z2 the compressibility coefficient at the end of the pipe. Z is determined by,

$$Z = 1 + \frac{9}{128} \frac{P_r}{T_r} (1 - 6T_r^{-2}) \quad (\text{Eqn 7})$$

where pr is the reduced pressure, Tr is the reduced temperature.

- 5 Based on equation 5, and with a low optimised frequency of 0.145 cycles/sec, or a period of 6.88 sec, air delay is not a factor.

### Fluidic Resistance

- Some people have found the fluidic resistance of air to have an affect on  
 10 the flowrate as it moves through the microchannels. This can be calculated from equations 8 and 9 and the effect on pressure compared to the pressures set in equation 5. To obtain the optimal inlet pressure, a loop can be set up between equation 5 and 8. The inlet pressure can then be adjusted in equation 5, which will alter the value of G, and therefore  
 15 Q, which will affect the inlet pressure in 8. The optimal inlet pressure is obtained when the values correspond in both equations.

The applied hydrodynamic pressure is the function of pumping rate and fluidic resistance as shown below.

$$\Delta p = R_f \times Q \quad (\text{Eqn 8})$$

where  $\Delta p$ ,  $R_f$  and  $Q$  denote the pressure difference, the fluidic resistance and the pumping rate, respectively. For a rectangular channel, the fluidic resistance is given by,

$$R_f = 12 \frac{\eta L}{wh^3} \quad (\text{Eqn 9})$$

- 25 It was found that p1 from equation 5 and 8 give the same value, proving that the fluidic resistance is negligible.

The desired flow rate, of 100  $\mu$ l/day is so low that it avoids problems relating to time delays in the micropump. It is also assumed that at such a low flow rate, the effects of shear stress on the cells will be minimal or non existent. The equipment has been designed to allow for the flow rates

5 to be altered and so if desired, further work could be conducted into the effects that increasing or decreasing rates may have.

## CLAIMS

1. A reactor comprising a platform, the platform at least defining:
  - at least one fluid reservoir arranged to receive fluid;
  - 5    -a plurality of reaction chambers, wherein the reaction chambers are discrete from each other;
  - a plurality of supply channels for transporting fluid from the at least one fluid reservoir to one or more of the plurality of reaction chambers;
  - 10    -pumping means arranged to pump fluid through one or more of a plurality of supply channels.
2. The reactor of claim 1, wherein the only external connection is an air/gas supply for the pumping means.
- 15    3. The reactor of claim 1 or claim 2, wherein, the platform further defines at least one outlet channel extending from at least one of the reaction chambers.
- 20    4. The reactor according to claim 3, wherein the platform further defines at least one collection reservoir arranged to collect fluid flowing from the at least one outlet channel.
- 25    5. The reactor according to any preceding claim, wherein the platform comprises a first layer, a second layer, and a third layer.
6. The reactor according to claim 5, wherein the reaction chambers and/or fluid reservoir and/or collection reservoirs are defined by the first layer.

7. The reactor according to claim 5 or claim 6, wherein the outlet channel(s) are defined by the first layer.
8. The reactor according to any of claims 5 to 7, wherein the second layer  
5 defines the supply channel(s)
9. The reactor according to any of claims 5 to 8, wherein the third layer may define the pumping means.
- 10 10. The reactor according to any preceding claim, wherein the supply channel(s) and the outlet channel(s) are not on the same plane.
11. The reactor according to any preceding claim, wherein the reaction chambers are in the form of discrete wells.  
15
12. The reactor according to any preceding claim, wherein the platform is arranged such that, in use, liquid does not flow, or perfuse from one reaction chamber to another reaction chamber.
- 20 13. The reactor according to any preceding claim, wherein each reaction chamber is individually accessible.
14. The reactor according to any preceding claim, wherein the reaction chambers are open-wells formed in the top-surface of the platform.  
25
15. The reactor according to any preceding claim, wherein the reaction chambers are capable of retaining a cell culture.
16. The reactor according to any preceding claim, wherein the platform  
30 comprises a transparent material.

17. The reactor according to any preceding claim, wherein the platform comprises a flexible material.

18. The reactor according to any preceding claim, wherein the platform  
5 comprises an air permeable material.

19. The reactor according to any preceding claim, wherein the platform is sized substantially the same as an industry/research standard microplate plate.  
10

20. The reactor according to any preceding claim, wherein the pumping means is physically separated from the supply channels by a wall or membrane.

15 21. The reactor according to any preceding claim, wherein the pumping means is a peristaltic membrane-based pneumatic micropump.

22. The reactor according to any preceding claim, wherein the platform further define at least one pump inlet aperture for connecting the at least  
20 one pump channel to an external air/gas supply, wherein the at least one pump inlet aperture is defined in the top-surface of the platform.

23. The reactor according to any preceding claim, wherein the bioreactor comprises a removable lid arranged to fit over the platform.  
25

24. The reactor according to any preceding claim, wherein the platform comprises means for heating or cooling the platform.

25. A method of manufacturing a reactor comprising the steps of:  
30 -forming a first layer, the first layer defining, at least in part, a plurality of reaction chambers, and at least one fluid reservoir;

-forming a second layer, the second layer defining at least one supply channel;

-forming a third layer, the third layer defining pumping means;

-assembling the second and third layer together, and assembling the  
5 first layer with either the second layer or the third layer to form a platform comprising a first, second and third layer.

26. The method according to claim 25, wherein at least one of the first, second and third layers comprises a flexible polymer.

10

27. The method according to claim 26, wherein the flexible polymer comprises at least one of the polymers selected from polydimethylsiloxane (PDMS), polypropylmethyilsiloxane (PPMS), polytrifluoropropylmethyilsiloxane (PTFPMS), and  
15 polyphenylmethyilsiloxane (PPHMS).

28. The method according to any of claims 25 to 27, wherein the at least one supply channel and/or the pumping means is provided by soft lithography.

20

29. The method according to any of claims 25 to 28, wherein the first layer is formed by moulding.

30. The method according to any of claims 25 to 29, wherein the first  
25 layer further defines at least one outlet channel provided by moulding.

31. Use of the reactor according to any of claims 1 to 24 for at least one of cell culture and cell analysis.

30 32. A kit comprising the reactor according to any of claims 1 to 24 and instructions.

33. A reactor substantially as described herein with reference to the description and the drawings.

- 5 34. A method of manufacturing a reactor as substantially described herein with reference to the description and the drawings.

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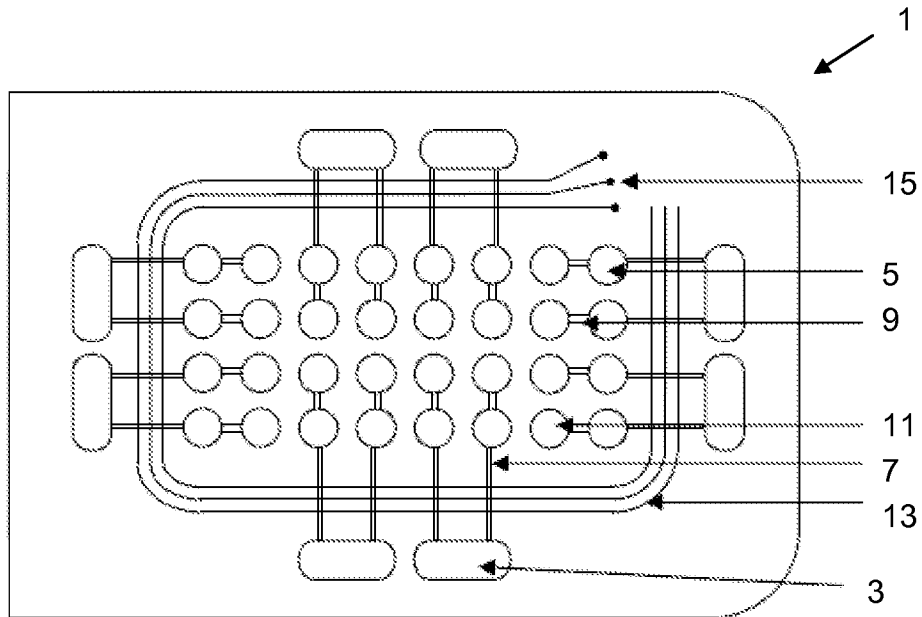


Figure 1

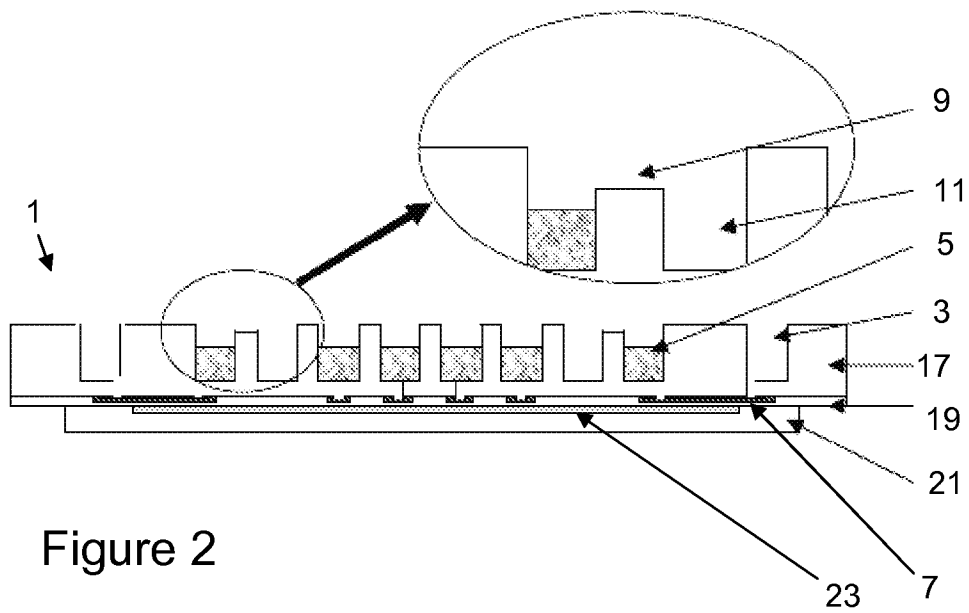


Figure 2

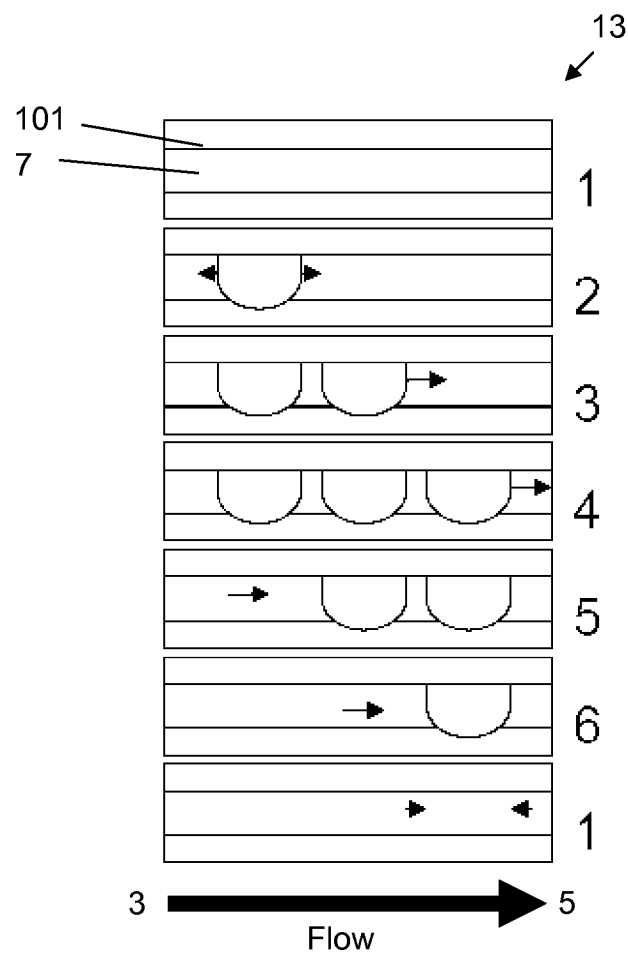


Figure 3

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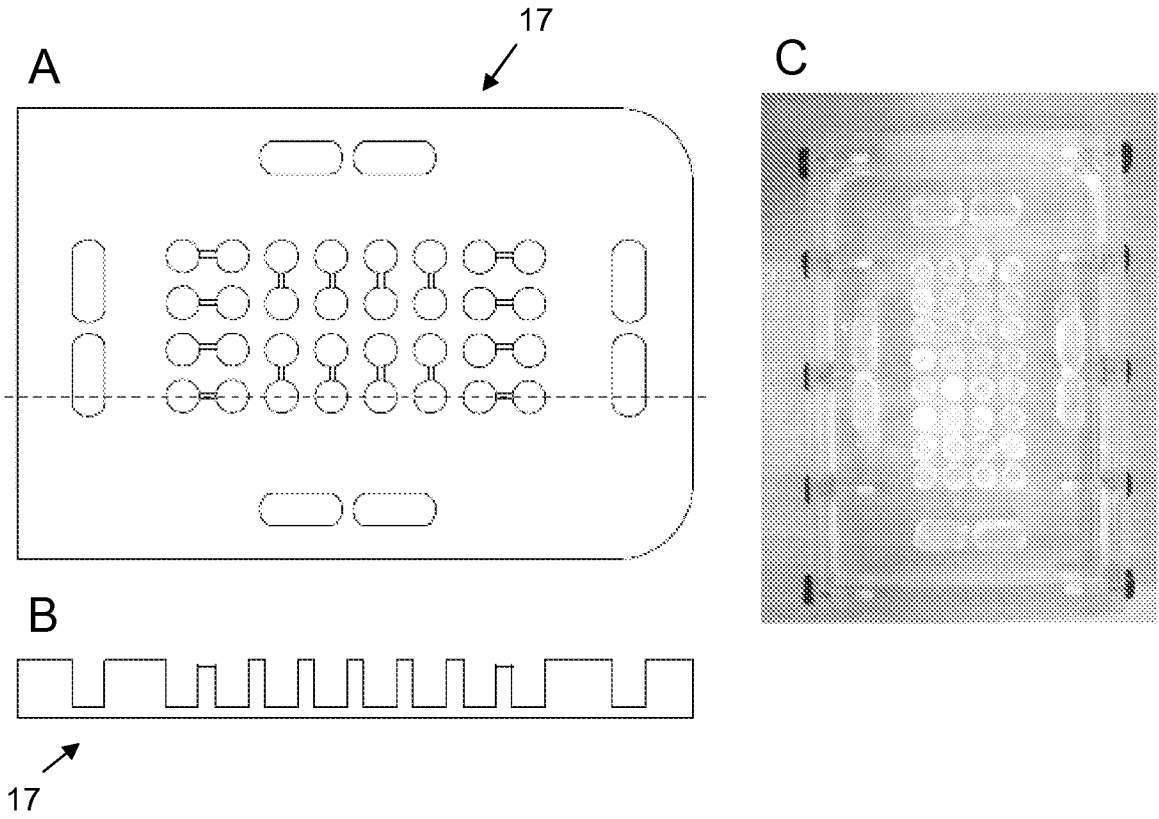


Figure 4

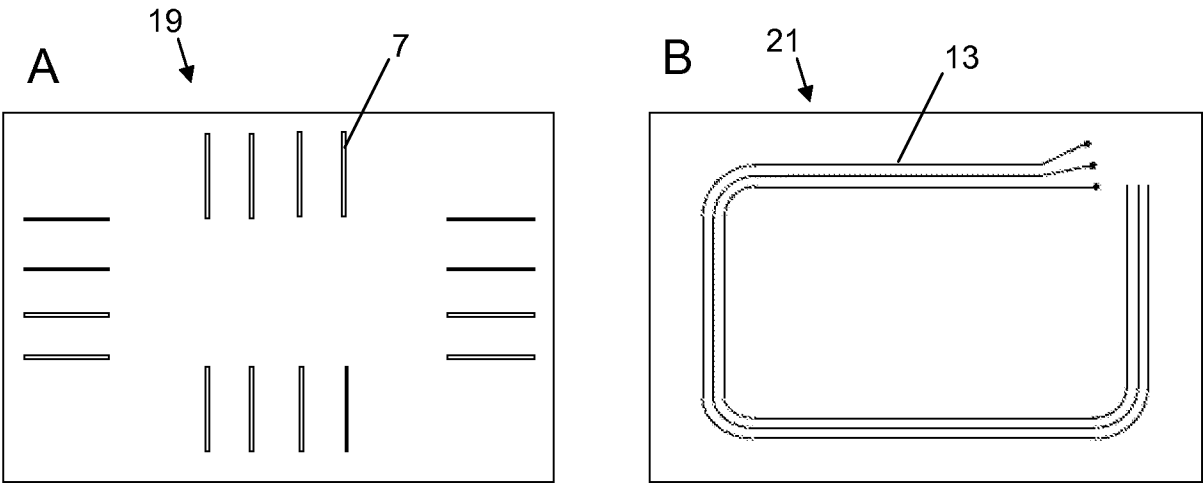


Figure 5

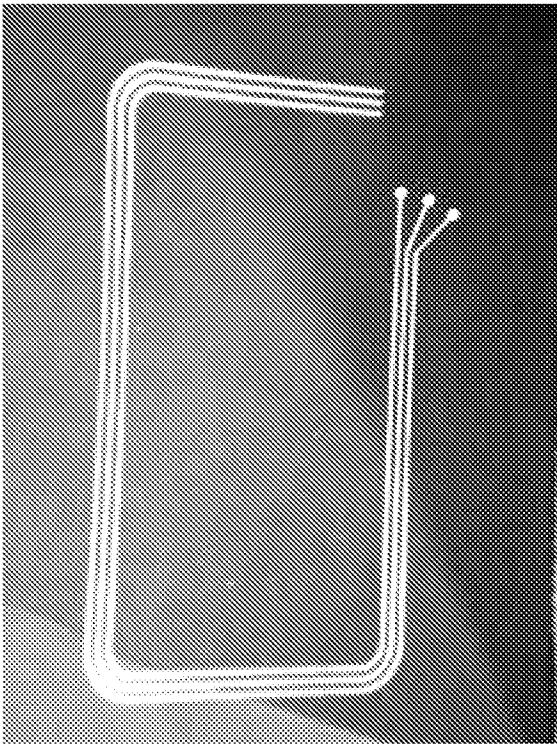


Figure 6

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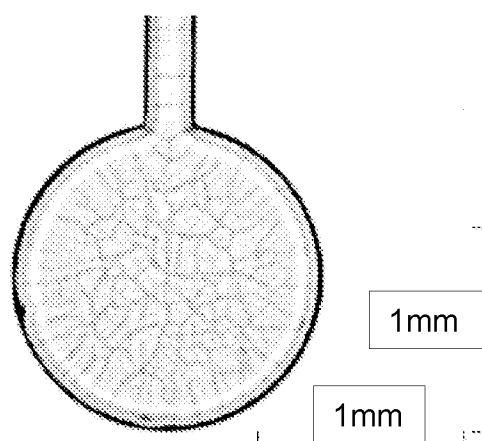


Figure 7

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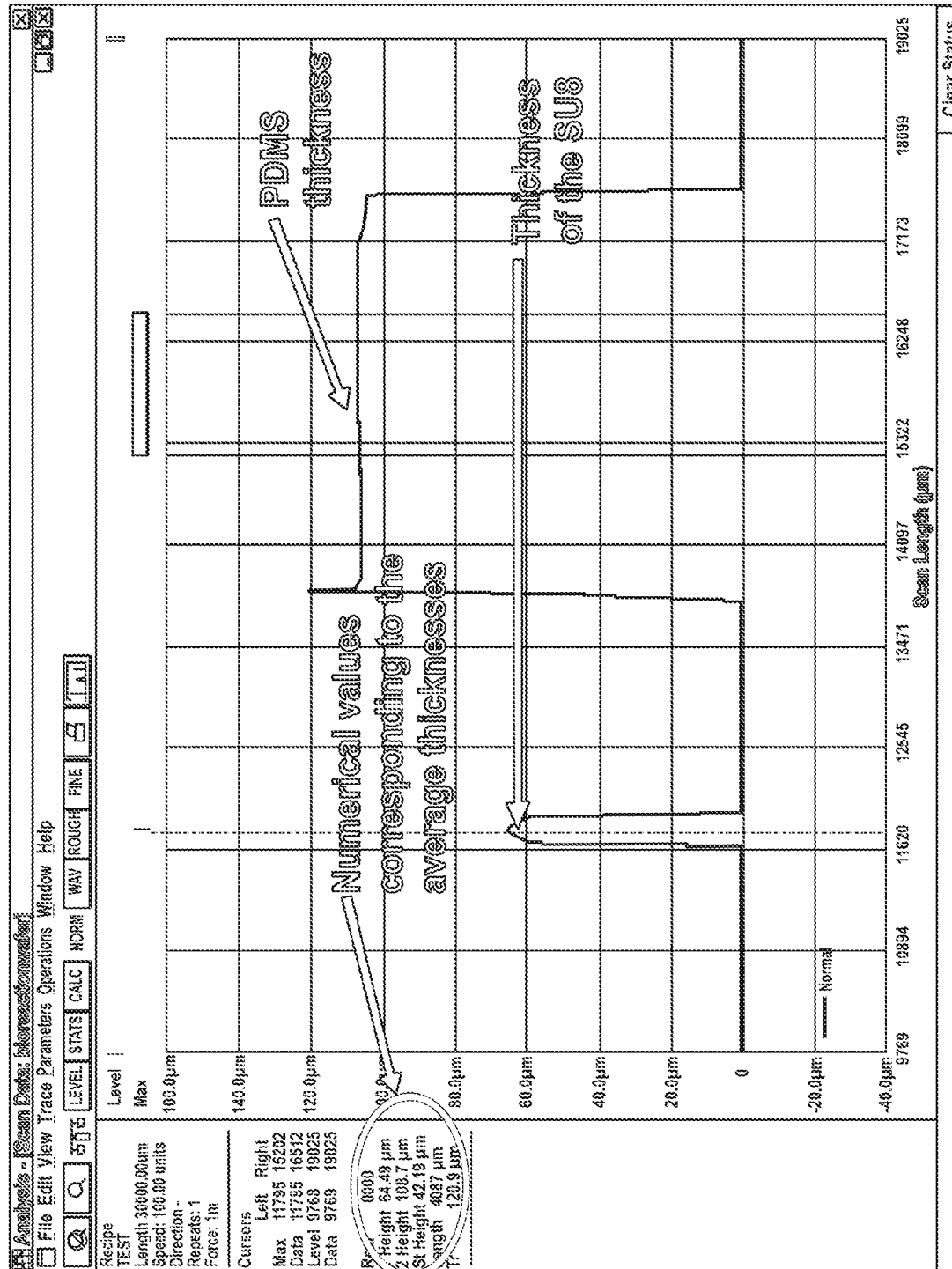


Figure. 8

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Figure 9

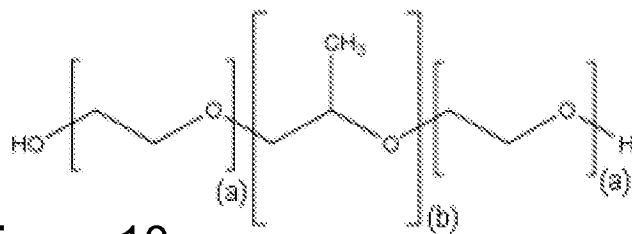


Figure 10

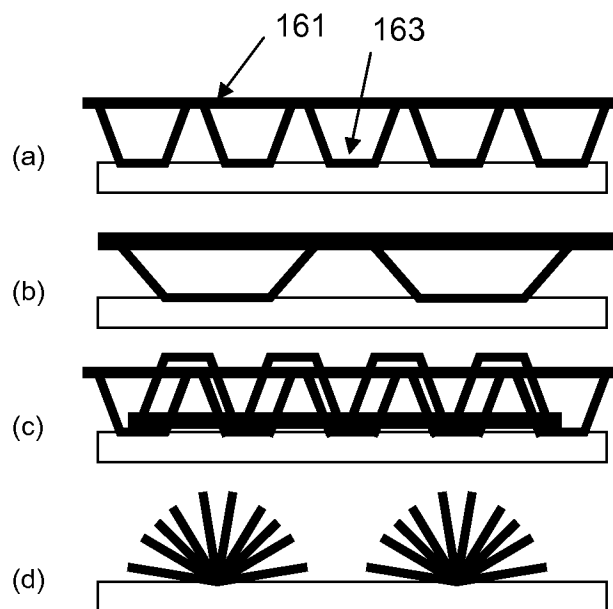


Figure 11

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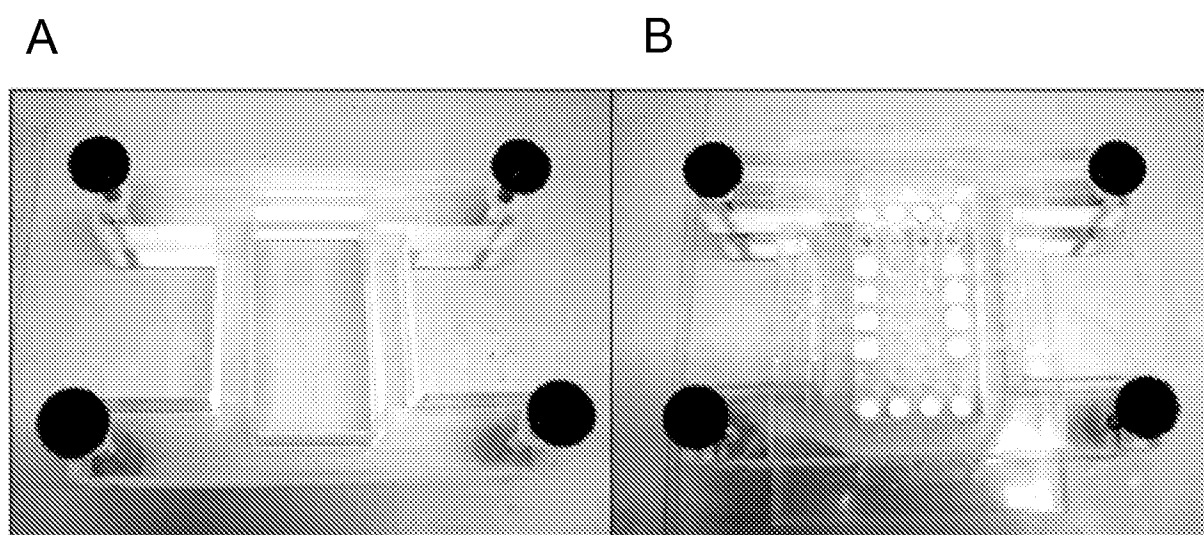


Figure 12

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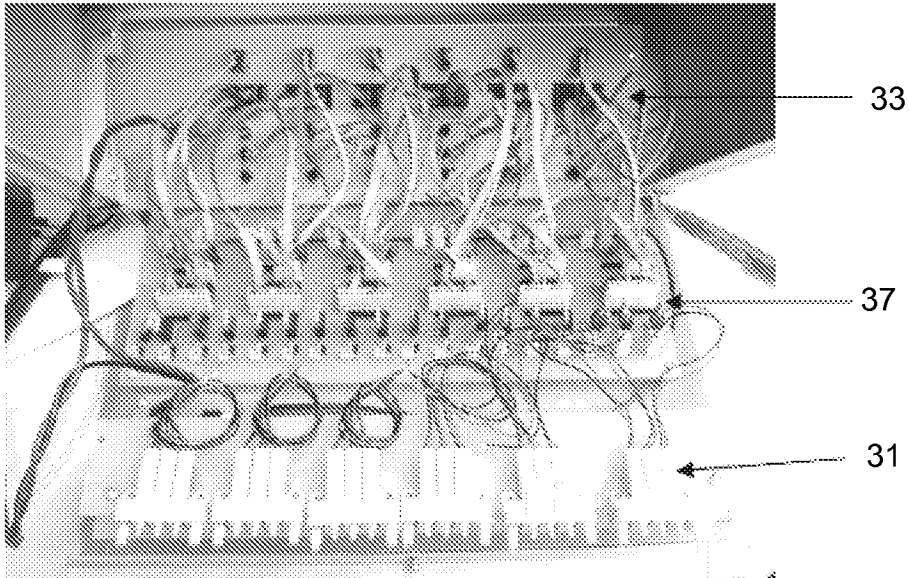


Figure 13

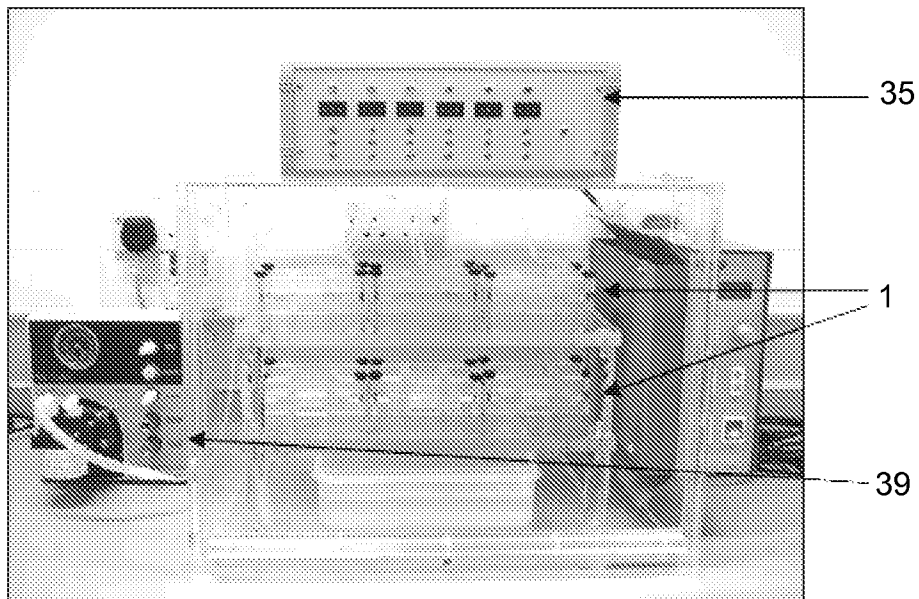


Figure 14

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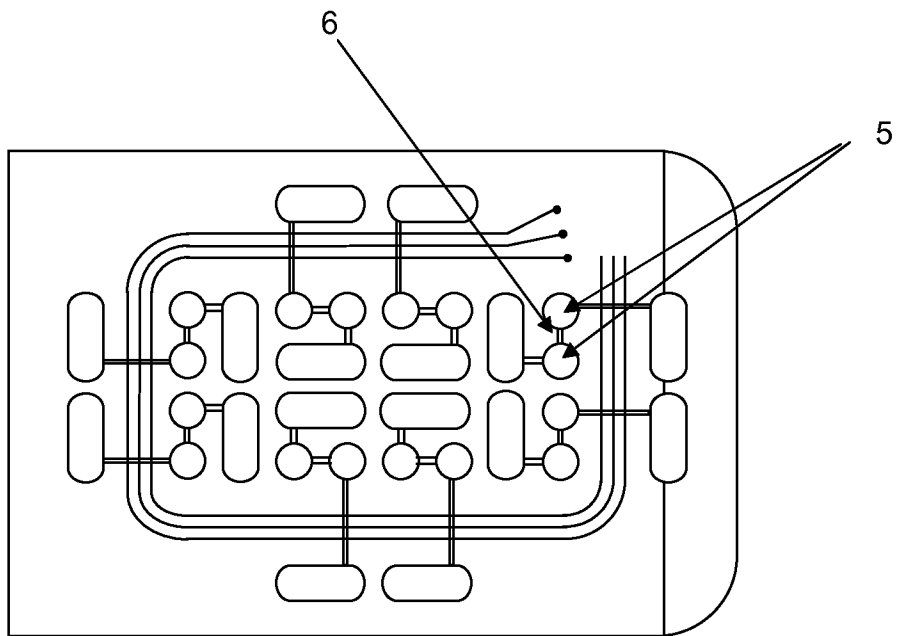


Figure 15