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(54) **Versatile 3D Stretchable Micro-Environment for Organ-on-Chip Devices Fabricated with Standard Silicon Technology**

(57) The present invention is in the field of microfluidic devices produced with silicon technology wherein at least one 3D microenvironment is present, a method of producing said device using silicon based technology, and a use of said device in various applications, typically a biological cell experiment, such as a cell or organ on a chip experiment, and use of the device as a microreactor.

Title Versatile 3D Stretchable Micro-Environment for Organ-on-Chip Devices Fabricated with Standard Silicon Technology

FIELD OF THE INVENTION

The present invention is in the field of microfluidic devices produced with silicon technology wherein at least one 3D microenvironment is present, a method of producing said device using silicon based technology, and a use of said device in various applications, typically a biological cell experiment, such as a cell or organ-on-a-chip experiment, and lab-on-a-chip experiment, and use of the device as a microreactor.

BACKGROUND OF THE INVENTION

A microfluidic device relate to a set of technologies with an aim to manipulate at least one small fluid (liquid or gas) volume within microsystems produced by human beings. In the device a cell culture or an individual cell or the like may be present. An experiment on said cell culture refers to the maintenance and growth of cells in a well-controlled environment. The environment may resemble naturally occurring circumstances. As such a cell can likewise be studied under application of at least one of numerous signals that might be present in their naturally occurring surrounding microenvironment.

A microfluidic cell culture may attempt to manipulate cells, such as by culturing, maintaining, and growing, and qualitatively and quantitatively experimenting and analyzing cells in microfluidic volumes. Such may relate to an attempt to understand a cell culture, such as a stem cell culture, non-dividing or slowly dividing cells, e.g. in terms of an interaction between cell culture parameters and the micro environmental conditions created by microfluidic devices. It is considered that dimensions of the microfluidics, such as chamber and channels, are well suited to the physical scale of the biological cells and other applications.

In general it is considered that microfluidics provide a good degree control over e.g. cell culture conditions. Typically a movement of fluids in the microfluidics is considered to be laminar; a fluid volume is typically in the order of 10^{-6}-10^{-12} l; fluid flow may be controlled precisely in terms of volume and timing, such as by providing an in-chip valve; also

precise chemical and physical control of the microenvironment is possible; a production of a multitude of individually controllable cell culture chambers on a single device is considered, albeit typical prior art technologies rely on manual procedures which are considered to be insufficiently controlled.

Some prior art documents recite microfluidic devices. WO2016/049363 A1, WO2016/049365 A1, WO2016/010861 A1, WO2016/004394 A1, and US15/2955534 A1 recite relatively simple organ on chip devices, which cannot include any complex sensing/stimulation elements; hence these device are not unsuited for most applications.

Huh, Dongeun, et al. In "Reconstituting organ-level lung functions on a chip." Science 328.5986 (2010): 1662-1668, Kim, Hyun Jung, et al. in "Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow." Lab on a Chip 12.12 (2012): 2165-2174, Huh, Dongeun, et al. in "Microfabrication of human organs-on-chips." Nature protocols 8.11 (2013): 2135-2157, and WO2015/138034 A1 present devices that have a limited production yield, limited throughput and limited functionality and do not seem to relate to more than a specific microfluidic device; the devices are considered to provide some basic functionality but are not ready in technological terms for further application.

The present invention relates to a device and a method of producing said device which overcomes one or more of the above or further disadvantages, without jeopardizing functionality and advantages.

SUMMARY OF THE INVENTION

The present invention relates in a first aspect to a device according to claim 1, which has amongst others the advantages of a higher throughput, being cheaper to produce, being more reliable and more versatile, providing a better handling of e.g. cells, and providing a wider functionality. For an organ-on-Chip the present device improves disease modelling, drug screening and toxicity tests. Pharmaceutical companies may use this as a tool to partially

replace animal testing improving animal welfare and drug testing reliability.

The present device comprises at least three distinct layer in which microfluidic and nano-/microscale elements and the like are provided. The first two layers are made of a polymer, typically but not necessarily the same polymer for both layers; a first polymer layer 20a is provided on a substrate, typically silicon 10 or a glass wafer, and is relatively thin; for the purpose of the invention the terms “substrate”, “silicon”, and “glass” are considered interchangeable; the top layer may be considered to relate to a membrane, which is considered to relate to a selective barrier; the top layer preferably is provided with a matrix of holes 28 therein, the at least one hole allowing passage of e.g. fluids, gases, species, microparticles, ions, etc. which can be adapted for specific uses; the top layer has a thickness of 0.05-30 µm, preferably 0.1-25 µm, more preferably 0.2-20 µm, even more preferably 0.5-8 µm thin, such as 1-5 µm or 2-3 µm; in contact with the relative thin polymer layer 20a is a thicker polymer bottom layer 20b; the bottom layer comprises at least one second micro-channel 21 and/or at least one second micro-chamber 22 at least partly embedded in the polymer bottom layer; the number, layout, sizes, and further characteristics of these microfluidics can be adapted for specific uses; the microfluidics may be embedded fully in the bottom layer 20b and/or may be embedded partly, such as in the case of a well; the polymer bottom layer is thicker than the top layer and preferably has a thickness of 50-2000 µm, hence is at least one order of magnitude thicker than the top layer, and typically 2-3 orders of magnitude thicker; the thickness is preferably 150-1000 µm, more preferably 200-500µm, even more preferably 250-400 µm; the device further comprises silicon based microfluidics in microfluidic contact with the top layer 20a of the polymer based microfluidics wherein the silicon based microfluidics are accessible and/or can be made accessible for use of the device; the substrate, e.g. silicon, based microfluidics comprise at least one first micro-channel 11 and/or at least one first micro-chamber 12 at least partly embedded (see above) in

the silicon, and at least one input 16, wherein the input 16 is in microfluidic contact with the at least one second micro-channel 21 and/or at least one second micro-chamber 22 embedded in the polymer bottom layer, e.g. as functionally defined or required; the support or substrate 10 may relate to a typically used wafer in a silicon semiconductor process such as of Si or glass; wherever silicon is mentioned in this respect it may relate to any other suitable substrate; the polymer top layer 20a is for separating (fluidics in the) at least one of the first micro-channel 11 and/or at least one of the first micro-chamber 12 embedded in the substrate (silicon) from (fluidics in the) at least one of the second micro-channel 21 and/or at least one of the second micro-chamber 22 embedded in the polymer bottom layer preferably at least partly by the matrix of holes 28 therein; the microfluidics of the polymer and silicon are directly or indirectly in microfluidic contact with one and another. The present polymer is independently selected from biocompatible polymers, such as polysiloxanes, such as polydimethylsiloxane (PDMS), polyimides, polyurethane, styrene-ethylene-butylene-styrene (SEBS), polypropylene, polycarbonate, polyester, polypropylene, and butyl rubber, and from biodegradable polymers, such as Biorubber (PGS), and poly(1,8-octanediol-co-citrate) (POC), and combinations thereof.

The term “fluidics” may relate to a gas, a liquid; and combinations thereof; a “microfluidic” is considered to be fluid under boundary conditions of the device.

The set-up composed by the polymer layers, typically forming a membrane, the micro-channels, the micro-chambers, and first micro-chamber (also referred to as “macro-chamber”) can be optically monitored off-line with a microscope and/or a camera e.g. placed on a backside/front-side of the device. The set-up can be monitored on-line by means of micro-electrode array and/or micro-fabricated sensors (such as flow/temperature/pH sensor) placed in the micro-environment and or in the macro-chambers. The set-up can be also altered/stimulated by means of liquid flow flowing through the micro-chamber/channels and the macro-chamber; likewise by gas flow flowing through the micro-chamber/channel and the macro-chamber; by

pressure differences applied in the micro-chambers, in the micro-channels and on the backside and the front side of the membrane (see e.g. fig. 11); by electrical stimulation provided by means of microelectrode arrays; by optical stimulation provided with optical systems placed on the backside/front-side of the device; by chemical stimulation provided by means of liquid flow or liquid reservoir placed in the membrane; and other micro-fabricated actuators placed inside the micro-channel/chambers; and combinations thereof, hence the device is considered to be versatile.

The presented microchip is typically fabricated on a Silicon substrate with standard IC and MEMS technologies. The silicon bulk and the chosen fabrication process provide advantages such as: use of standard cleanroom compatible micro-fabrication processes to achieve wafer-scale fabrication that can be scaled up to volume production by eliminating manual assembly and sample handling. This enables high yield and throughput, thus, low cost volume production. The device can be equipped with additional modules embedded on the backside and/or front-side of the membrane and/or in the microenvironment composed by the micro-channels/chambers. By adding or removing one or more modules during fabrication, it is possible to adapt the device to meet different demands. Examples of the possible modules are: a micro-electrode array for electrical stimulation/monitoring of tissues in vitro cultured in the environment and or the gasses/liquid in the environment; a reference electrode for cell culture monitoring;

Flow/temperature/pH sensors and or strain gauges for monitoring the environment in the micro-chamber/channels and/or macro-chambers; IC circuits such as pre-amplifiers for the signal detected by the sensors mentioned above; Micro-grooves to promote cell alignment which grooves may be coated such as with an adhesive molecule and/or elastomer; micro-pillars to mix fluids; Micro-fabricated actuators such as hot-plate to regulate environment temperature.

The present device can be used for various application including e.g. study of growth and differentiation of primary cells, such as human neuronal cells as well as any other cell requiring e.g. mechanical and/or electrical stimu-

lations and also stratified structure; simulation of a micro-environment in a living tissue and/or organ.

The present device may find numerous applications in various Organ-on-Chips, Lab-on-Chips, microfluidics, and microreactors. The capability of the present device of having integrated electrical microstructures 29,90 allows to have cell micro-environments where real-time monitoring and stimulating of different types of cells is possible; e.g. electrical stimulation of heart cells or neurons, reading of neuron and heart cells electrical activity, transendothelial electrical resistance measurements in brain-blood barrier models, among other applications.

The present device also allows the control over the mechanical and topographical signals supplied to a cell micro-environment through its configurable polymeric layers, e.g. patterned surface 27, to improve adhesion and alignment of heart cells, flexible membrane layers 20a,b to apply stress on cultured cells locally, microporous surface 28 to allow and study migration of immune cells in lung and/or skin models, and study of the interface between two different cell cultures. Additionally, the device presents micro-features to precisely supply cell microenvironments with controlled fluid flows to allow air flow and/or blood flow in lung, liver, gut or brain barrier models, and cell microenvironment with different drugs and micro-chambers 22 that can be used as reservoirs for this drugs and/or any other biological or chemical agent. Therefore, the present device is adaptable to develop at least one of several organ-on-chip platforms such as Brain-on-Chip, Heart-on-Chip, Lung-on-Chip, Gut-on-Chip, Blood-Brain Barrier-on-chip, Liver-on-Chip and/or Kidney-on-Chip.

On the other hand, the inlets, microchannels and microchambers of the present device may be used to develop microfluidics devices and/or microreactors either on a substrate to study biological processes and/or other phenomena requiring a precise control over the flow conditions in a micron and/or millimeter scale environment.

In a second aspect the present invention relates to a method of producing the present device. Therein e.g. a Si substrate 10 is provided and thereafter various more or less

standard semiconductor process steps may be performed; first a first dielectric layer (51a,b) is deposited/grown on both sides of the substrate, and thereafter the dielectric layer is patterned on a bottom side; then depositing a first membrane layer 61 of polymer material on the dielectric layer; the membrane layer is typically spun on the unpatterned Si-side of the dielectric layer, in an example PDMS is spun; thereafter a patterning step of the first membrane layer 61 is performed using optical lithography or electron beam lithography; it is considered quite atypical to use such patterning tools, as typically soft-lithographic process are used, that is "by hand"; in addition prior art processes are typically not fully integrated, such as being compatible with semiconductor processes; thereafter a sacrificial layer 72 is deposited on the first membrane layer, such as a dielectric layer or a photo resist (PR) layer; the PR may be provided by spinning; hereafter the first membrane layer 61 is patterned using a lithography or electron beam machine; typically an alignment step is involved for aligning microscopic/nanoscopic features; then a second membrane layer 62 is deposited on the sacrificial layer 72, such as by spinning; the second membrane layer may be of the same material (polymer) as the first membrane layer 61, or may be of a different material; then the Silicon substrate 10 is etched , preferably using dry etching, at the bottom side thereof, preferably stopping etch on the first dielectric layer, therewith providing openings for channels 11/chambers 12 in Si; the first dielectric layer 51b is then (wet) etched from the bottom side providing openings for channels/chambers; and as a further step the sacrificial layer 71 is then (wet) etched thereby releasing channels 21/chambers 22. The wafer may then be diced (cut) and mounted on an assembly including e.g. a well 35 for cell culturing, a microfluidic inlet 32, a pneumatic inlet to stretch the membrane 36 and an electrical output to read the output of the electrodes, and the sensors embedded in the chip 38. The present method therewith provides a way of producing the present device.

In a third aspect the present invention relates to a use of the present device for at least one of a biological cell experiment, organ on a chip experiment, an optical

microscope experiment, growth and differentiation of primary cell experiment, such as a human neuronal cell, mechanical and electrical stimulation of a cell, a stratified structure, simulation of a microenvironment in living tissue and/or organ, as Lab-on-Chip, as a microfluidics device, and as a micro-reactor; hence the present device may be considered to be very versatile.

It is noted that some of the steps may be performed in a different sequence, and/or at a later or earlier stage.

Thereby the present invention provides a solution to one or more of the above mentioned problems.

Advantages of the present invention are detailed throughout the description.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates in a first aspect to a device according to claim 1.

In an example the present device further comprises at least one of a microchip, an integrated sensor, and an output 18. The microchip may be fully integrated in the silicon (10), such as in a silicon substrate. The microchip may perform control functions and process input and provide (data) output. The output 18 may be located in the silicon and/or in the polymer; more than one output as well as more than one input may be present. The integrated sensor may be provided in the silicon and may be silicon based; the sensor is typically provided in a microfluidic channel/chamber. The sensor may relate to a chemical sensor, a physical sensor, etc.

In an example of the present device the membrane is stretchable having a tensile strength of > 1 [MPa] (ISO 527) and/or flexible with a Young's modulus of <3 [GPa] (ISO 527), or wherein the membrane is rigid having a Young's modulus of >10 [GPa] (ISO 527).

In an example of the present device the polymer is independently selected from biocompatible polymers, such as polysiloxanes, such as polydimethylsiloxane (PDMS), polyimides, polyurethane, styrene-ethylene-butylene-styrene (SEBS), polypropylene, polycarbonate, polyester, polypro-

pylene, butyl rubber, and biodegradable polymers, such as Biorubber (PGS) and poly(1,8-octanediol-co-citrate) (POC), and combinations thereof. It may be an advantage to use a biodegradable material, as e.g. after initial culturing an interface between two cultures may gradually disappear. The polymer may be porous or non-porous, thereby having a certain permeability to fluids, such as determined by ISO 15105-1 or ISO 2556 for a gas and ISO 2528 for water.

In an example of the present device the membrane comprises an array of nxm openings, wherein $n \in [1,10^6]$, preferably $n \in [2,10^5]$, more preferably $n \in [5,10^4]$, even more preferably $n \in [10,10^3]$, such as $n \in [100,500]$, wherein $m \in [1,10^6]$, preferably $m \in [2,10^5]$, more preferably $m \in [5,10^4]$, even more preferably $m \in [10,10^3]$, such as $m \in [100,500]$, wherein a density of holes is 0.001-250/100 μm^2, preferably 0.01-100/100 μm^2, more preferably 0.1-50/100 μm^2, even more preferably 1-20/100 μm^2, and/or wherein an average hole area is 0.05-500 μm^2, preferably 0.1-200 μm^2, more preferably 0.2-100 μm^2, even more preferably 0.5-50 μm^2, such as 1-5 μm^2 or 10-30 μm^2.

In an example the present device further comprises embedded in the device at least one of a sensor, a pump, a microelectrode, a valve, a strain gauge, an actuator, a heater, a cooler, a stimulator, a flow sensor, a temperature sensor, a pH sensor, an IC-circuit, an amplifier, an actuator, a hot plate, a micro-electrode array, a chemical stimulator, an optical stimulator, a pressure regulator, an ion sensor, and further microfluidic elements. Such expresses the versatility of the present design and manufacturing method.

In an example the present device further comprises embedded in the thin polymer top layer 20a at least one electrode 29 and a microgroove, wherein the electrode preferably has an accessible area 29a of 0.2-5000 μm^2, preferably 0.25-2500 μm^2, more preferably 0.5-2000 μm^2, even more preferably 1-1000 μm^2, such as 2-500 μm^2 or 5-100 μm^2; the microgrooves may have a length between 0.4 to 5000 µm, such as 1 to 500 µm, a width between 0.4 to 5000, such as 1 to

50 µm, and a depth between 0.2 and 50 µm, such as 1 to 20 µm, e.g. 2 to 5 µm.

In an example of the present device the thin polymer top layer 20a comprises at at least one side thereof, at least one micro-feature, such as an indentation, a groove, a topographical structure, preferably at least one oriented microgroove, preferably an array of x*y oriented microgrooves, wherein $x \in [1,10^6]$, preferably $x \in [2,10^5]$, more preferably $x \in [5,10^4]$, even more preferably $x \in [10,10^3]$, such as $x \in [100,500]$, wherein $y \in [1,10^6]$, preferably $y \in [2,10^5]$, more preferably $y \in [5,10^4]$, even more preferably $y \in [10,10^3]$, such as $y \in [100,500]$,wherein a density of microgrooves is 10^{-4}-25/100 μm^2, preferably 10^{-3}-10/100 μm^2, more preferably 10^{-2}-5/100 μm^2, and/or wherein an average groove area is 0.1-10^6 μm^2, preferably 1-10^5 μm^2, more preferably 10-10^4 μm^2, even more preferably 100-10^3 μm^2, such as 200-500 μm^2, and/or wherein a groove length is from 5 µm-5 mm, and/or wherein the at least one micro-feature is aligned with respect to the device.

In an example of the present device at least one of the first micro-channel 11 and/or at least one of the first micro-chamber 12 embedded in the substrate (silicon) is accessible from the outside (i.e. “partly open”), and/or wherein the at least one of the first micro-channel 11 and/or at least one of the first micro-chamber 12 embedded in the substrate (silicon) have a height of 50-2000 µm, preferably 100-1000 µm, more preferably 200-500 µm, such as 300-400 µm; the microchamber 12 and the microchannel 11 may have various shapes, selected from circular, rectangular, hexagonal, oval, and multigonal; the microchannel may have an area of 20-10^6 μm^2, preferably 100-10^5 μm^2, more preferably 400-10000 μm^2; the at least one of the second microchannel 21 and/or at least one of the second micro-chamber 22 embedded in the polymer have a height of 1-1000 µm, preferably 50-500 µm, more preferably 100-400 µm, such as 200-300 µm.

In an example of the present device the first micro-channel (11) comprises at least one column made of polymer, which connects the top and the bottom side of the

channel, preferably at least one oriented column, preferably an array of c*d columns 26, wherein $c \in [1,10^6]$, preferably $c \in [2,10^5]$, more preferably $c \in [5,10^4]$, even more preferably $c \in [10,10^3]$, such as $c \in [100,500]$, wherein $d \in [1,10^6]$, preferably $d \in [2,10^5]$, more preferably $d \in [5,10^4]$, even more preferably $d \in [10,10^3]$, such as $d \in [100,500]$, wherein a density pillars is 10^{-4}-25/100 μm^2, preferably 10^{-3}-10/100 μm^2, more preferably 10^{-2}-5/100 μm^2, and/or wherein a section area of a pillar is 1-10^7 μm^2, preferably 10-10^6 μm^2, more preferably 100-10^5 μm^2, even more preferably 1000-5*10^4 μm^2, such as 1000-10^4 μm^2.

In an example of the present device the walls of the micro-chamber 21 may be coated with a conductive material 14, such as platinum, or with an electrically insulating material, such as parylene, or a combination of both. The platinum coating may be used as additional electrode directly in contact with the reservoir 35.

In an example of the present device the polymer layers 20a,20b are provided with openings, the openings providing access to at least one of a metal pad, an IC, a sensor, such as an optical sensor, a heater, etc.

In second aspect the present invention relates to a method according to claim 11.

In an example of the present method the first and second dielectric layers 51a,b,52 are made from a material independently selected from Si-dielectric materials, such as SiO_2, and Si_3N_4.

In an example of the present method a thickness of the first 51a,b and second 51 dielectric layer are each independently from 5-500 nm, preferably 10-250 nm, more preferably 20-100 nm, such as 30-50 nm.

In an example of the present method the flexible and or stretchable second and third dielectric layers 52,53 are made from a material independently selected from polymers such as polyamide and parylene.

In an example of the present method a thickness of the first membrane layer (61) is from 50-30000 nm, preferably 250-5000 nm, more preferably 500-2000 nm, such as 1000-1500 nm.

In an example of the present method a thickness of the second membrane layer 62 is from 50-2000 µm, preferably 200-1000 µm, more preferably 300-800 µm, such as 500-700 µm.

In an example of the present method the membrane layers 61,62 are each independently made from a material selected from a biopolymer, preferably a biocompatible polymer, such as polysiloxane, such as PDMS, polyimides, parylene, and biodegradable polymers, such as Biorubber (PGS) and poly(1,8-octanediol-co-citrate) (POC), and combinations thereof.

In an example of the present method the sacrificial layer 72 is a photo resist, such as an I-line photo resist, silicon oxide, and a metal.

In an example of the present method patterning is performed using an I-line lithographic machine, such as an ASML PAS 5500.

In an example of the present method at least one dielectric layer is formed by one of PECVD, LPCVD, low-temperature PECVD, and thermal oxidation.

In an example of the present method dry etch of silicon is performed using DRIE and/or wherein wet etching of silicon is performed using KOH.

In an example of the present method at least one of the membrane layers is spun.

In an example the present method may comprise at least one step selected from:

providing a Si-substrate 10, optionally comprising at least one sensor 90,

a1) depositing/growing a first dielectric layer (51a,b) on at least one side of the substrate, and

a2) patterning the dielectric layer on top and bottom side;

b1) depositing a metal layer on the top side of the substrate;

b2) patterning the metal layer;

c1) depositing the first flexible and/or stretchable dielectric layer on the top side of the substrate;

c2) patterning the first flexible and or stretchable dielectric layer;

d1) depositing a conductive layer such as metal and/or conductive polymers) on the top side of the substrate;

d2) patterning the conductive layer;

e1) depositing the second flexible and/or stretchable dielectric layer;

e2) patterning the second flexible and or stretchable dielectric layer;

f1) depositing the first sacrificial layer for the first set of micro-grooves;

f2) patterning the first sacrificial layer 71;

g) depositing a first membrane layer 61 of the second dielectric layer; PDMS spinning on unpatterned Si-side

h) patterning the first membrane layer 61 using a lithography or electron beam machine;

i) partially etching the first membrane layer 61 using a lithography or electron beam machine for the second set of microgrooves

l1) depositing a second sacrificial layer 72 on the first membrane layer, such as PR, and PR spinning and

l2) patterning the second sacrificial layer 61 using a lithography or electron beam machine;

m) depositing a second membrane layer 62 on the sacrificial layer 72;

n) patterning the second membrane layer 61

o) dry or wet etching the Silicon substrate 10 at the bottom side, preferably stopping etch on the first dielectric layer, therewith providing openings for channels 11/chambers 12 in Si;

p1) deposition of a conductive and/or insulating chamber coating, such as platinum parylene ;

p1) etching of the conductive or insulating chamber coating;

q) (wet) etching of the first dielectric layer 51b from the bottom side providing openings for channels/chambers; and

r) (wet) etching of the sacrificial layer 72 thereby releasing channels 21/chambers 22.

In an example of the present method dimensions of the at least one first micro-channel 11 and/or at least one first micro-chamber 12 embedded in the substrate (silicon), the at least one input 16, the at least one second micro-channel 21 and/or at least one second micro-chamber 22 embedded in the polymer bottom layer, are each independently fully adaptable

in a range of 50 nm-2 mm, and/or wherein the dimensions of the matrix of holes 28, the micro-features, are each independently fully adaptable in a range of 50 nm-100 µm, such as by lithography, such as by E-UV-I-line lithography and/or by e-beam lithography.

In an example of the present method the substrate layer comprises at least two alignment markers, and wherein during at least one method step the substrate 10 is aligned.

In a third aspect the present invention relates to a use according to claim 25.

In an example of the present use a wet/humid section and a dry section of the device are physically separated, wherein the dry section comprises electronics.

An example of the present use is as a blood-brain barrier model. The model may comprise brain microvascular endothelial cells (BMEC) and astrocytes cultured in the microchannel 21 or microchamber 22. These cells may be cultured on the silicon microchamber 12 together with neuron cells and/or other brain cells. The membrane layer 61 with a patterned surface 27 represents in such a case a dynamic interface that separates a central nervous system from a circulation system and as such creates a barrier. The microchannel 21 then allows to generate and supply shear stress to the barrier having effect on its permeability and function. The shear stress might be generated by blood or gas flowing through the microchannel 21 supplied by the silicon and polymer inlets in microfluidic contact 11,21. The electrical microstructures 21,14,90 make it possible to have an integrated transendothelial electrical measurement (TEER). The polymer membrane 20a,b also allows mechanical stimulation of the interface of the said cultured microenvironment.

In a fourth aspect the invention relates to an assembly comprising at least one of the present device 100, a reservoir 35 comprising a chip and a cylinder 31, a sealing on top of the device, a pressure chamber 36 comprising an inlet 34, an electrical input/output 35 connected to a printed circuit board (PCB) with at least one electrical output 18, e.g. via flip chip connections or wirebonding connections 37, for interfacing with e.g. an electrode 29 and/or a sensor 90.

The invention is further detailed by the accompanying figures and examples, which are exemplary and explanatory of nature and are not limiting the scope of the invention. To the person skilled in the art it may be clear that many variants, being obvious or not, may be conceivable falling within the scope of protection, defined by the present claims.

FIGURES

Figures 1a-r show details of an exemplary embodiment of the present method.

Figs. 2a-d, 3ab, 4-9, 10a-c, 11a,c-12 show exemplary details of the present device.

DETAILED DESCRIPTION OF THE FIGURES

In the figures:

100	microfluidic device
10	substrate based microfluidics
11	first micro-channel
12	first micro-chamber (macro-chamber)
14	coating layer
16	input
18	output
20	polymer based microfluidics
20a	polymer top layer
20b	polymer bottom layer
21	second micro-channel
21b	isolated channel
21c	channel, such as for drug delivery
22	second micro-chamber
26	(array of) columns
27	patterned structure
28	matrix of holes
28a	single hole, such as for drug delivery
29	electrode
29a	accessible area of electrode
31	cylinder
32	microfluidic inlet
34	pneumatic chamber
35	reservoir
36	pneumatic inlet

37 electrical connection
38 electrical input/output
39 printed circuit board
51a,b first dielectric layer
52 second dielectric layer
53 third dielectric layer
61 first membrane polymer layer
62 second membrane polymer layer
71 first sacrificial layer
72 second sacrificial layer
81 contact pad
82 metal line and electrode
90 sensor

Figures 1a-r show details of an exemplary embodiment of the present method. The method includes fabrication of a microelectrode array, an array of columns in the channel, two set of microgrooves, and an embedded sensor/electrode in the Silicon support.

Figure 1a shows provision of a silicon substrate and Silicon Oxide deposition (front and back) and patterning (on Silicon wafer with integrated sensor)

Figure 1b shows Aluminum deposition and patterning (for contact pads)

Figure 1c shows First isolation layer (such as parylene/ polymide) deposition and patterning

Figure 1d shows Metal deposition and patterning (for metal lines and electrodes)

Figure 1e shows Second isolation layer (such as parylene/ polymide) deposition and patterning

Figure 1f shows Spinning and patterning of sacrificial layer (for first set of Micro-grooves)

Figure 1g shows PDMS spinning

Figure 1h shows PDMS patterning (landing on wafer)

Figure 1i shows PDMS patterning (partial etching - second set of grooves)

Figures 1la-c shows sacrificial layer deposition and patterning (for channels)

m) Second PDMS layer spinning

n) Second PDMS layer patterning

File: Draft3

o) Silicon etching

p) Macro-chamber coating (platinum/parylene)

q) Silicon oxide etching

r) Micro-chamber/channels releasing and first set of grooves releasing.

Figure 2a,b,c,d show details of an exemplary embodiment of one device that includes one channel 21 accessible through an inlet and an outlet 16 and through hole matrix 28 in the microchamber 12.

Figure 3a and 3b show respectively details of an exemplary embodiment of two devices equipped with a microchannel 21 and a microchamber 22 respectively embedded in the polymer layers 20a,b.

Figure 4 shows details of an exemplary embodiment of a device equipped with an array of electrodes embedded in the top polymer layer 20a.

Figure 5 shows details of an exemplary embodiment of a device with an etched hole in the top polymer layer 20a that expose the sensor/electrode 90 with the solution in channel 21.

Figure 6 shows details of an exemplary embodiment of a device with an opening on the top and bottom polymer layer than can be used as electrical input/output for the electrodes and/or sensors 28,90 and layer 14.

Figure 7 shows details of an exemplary embodiment of a device equipped with an array of microgrooves to promote the alignment of the cell culture in the reservoir 35.

Figure 8 shows details of an exemplary embodiment of a device equipped with an array of columns 26 connecting the two polymer layer 20a,20b separated by a microchamber 21 and or a microchannel 22.

Figure 9 shows details of an exemplary embodiment of a device equipped with a coating layer 14 deposited on the walls of the microchamber 12. This may be used as reference electrode in case of a conductive coating layer such as platinum or as an electrical isolation from the cell culture in case of an isolating layer such as parylene or polyamide.

Figure 10a,b,c, show details of an exemplary embodiment of one device that includes three independent channels 21 accessible through four inlets 16 and via hole matrix 28 in the microchamber 12. One of the channel 21a is connected to two inlet and may be used for 3D cell culturing. Channel 21c is connected to chamber 12 via a single hole 28a and may be used to deliver drugs to the cell culture. Channel 21b is isolated and can be used to locally stretch the cell culture in 12 by applying a difference in pressure between the channel 21c and the microchamber 12.

Figure 11 shows details of an exemplary embodiment of one device when it is in relaxed state (fig. 11a), when the polymer layers are stretched by applying a difference of pressure between the microchamber 12 and the back of the thick polymer layer 20b (fig. 11b), when the thin polymer layer is locally stretched by applying a difference of pressure between the microchamber 12 and the microchannel 12 (fig. 11c).

Figure 12 shows details of an exemplary embodiment of one device mounted in an assembly composed by or more device 100, one or more reservoir 35 composed by the chip and a cylinder 31 sealed on top of the device 100, one or more microfluidic inlet 32 to impose a flow in the channel 21, one or more pressure chamber 36 comprising an inlet 34 and one or more electrical input/output 38 placed on a printed circuit board 39 connected to the device electrical output 18 via flip chip connections or wirebonding connections 37, in order to interface with the electrodes 29 and or the sensors 90 embedded in the device 100.

EXAMPLES/EXPERIMENTS

The invention although described in detailed explanatory context may be best understood in conjunction with the accompanying examples and figures.

The following section is added to support the search to the prior art and it reflects the translation of the claims into English.

1. Micro-fluidic Device (100) comprising

(a) polymer based microfluidics (20), comprising

a 0.05-30 μm thin polymer top layer (20a) the polymer top layer preferably having a matrix of holes (28) therein,

a 50-2000 µm polymer bottom layer (20b) in contact with the polymer top layer, and comprising at least one second micro-channel (21) and/or at least one second micro-chamber(22) at least partly embedded in the polymer bottom layer,

(b) substrate based microfluidics (10) in microfluidic contact with the top layer (20a) of the polymer based microfluidics wherein the silicon based microfluidics are accessible and/or can be made accessible for use of the device, comprising

at least one first micro-channel (11) and/or at least one first micro-chamber(12) at least partly embedded in the substrate (silicon), and

at least one input (16),

the input (16) being in microfluidic contact with the at least one second micro-channel (21) and/or at least one second micro-chamber(22) embedded in the polymer bottom layer, and

the polymer top layer (20a) separating at least one of the first micro-channel (11) and/or at least one of the first micro-chamber(12) embedded in the substrate (silicon) from at least one of the second micro-channel (21) and/or at least one of the second micro-chamber (22) embedded in the polymer bottom layer preferably at least partly by the matrix of holes (28) therein.

2. Device according to claim 1, further comprising at least one of a microchip, an integrated sensor, and an output (18).

3. Device according to any of the preceding claims, wherein the membrane is stretchable having a tensile strength of > 1 [MPa] (ISO 527) and/or flexible with a Young's modulus of <3 [GPa] (ISO 527), or wherein the membrane is rigid having a Young's modulus of >10 [GPa] (ISO 527).

4. Device according to any of the preceding claims, wherein the polymer is independently selected from biocompatible polymers, such as polysiloxanes, such as polydimethylsiloxane (PDMS), polyimides, polyurethane, butyl rubber, styrene-ethylene-butylene-styrene (SEBS), polypropylene, polycarbonate, polyester, polypropylene, and biodegradable polymers,

such as Biorubber (PGS) and poly(1,8-octanediol-co-citrate) (POC), and combinations thereof.

5. Device according to any of the preceding claims, wherein the membrane comprises an array of nxm openings, wherein a density of holes is 0.001-250/100 μm^2, and/or wherein an average hole area is 0.05-500 μm^2.

6. Device according to any of the preceding claims, further comprising embedded in the device at least one of a sensor, a pump, a microelectrode, a valve, a strain gauge, an actuator, a heater, a cooler, a stimulator, a flow sensor, a temperature sensor, a pH sensor, an IC-circuit, an amplifier, an actuator, a hot plate, a micro-electrode array, a chemical stimulator, an optical stimulator, an ion sensor,
a pressure regulator, and further microfluidic elements.

7. Device according to any of the preceding claims, comprising embedded in the thin polymer top layer (20a) at least one electrode (29), wherein the electrode preferably has an accessible area (29a) of 0.1-5000 μm^2.

8. Device according to any of the preceding claims, wherein the thin polymer top layer (20a) comprises at at least one side thereof, at least one micro-feature, such as an indentation, a groove, a topographical structure, preferably at least one oriented microgroove, preferably an array of x*y oriented microgrooves, wherein a density of microgrooves is 1-25/100 μm^2, and/or wherein an average groove area is 0.1-10^6 μm^2, and/or wherein the at least one micro-feature is aligned with respect to the device.

9. Device according to any of the preceding claims, wherein at least one of the first micro-channel (11) and/or at least one of the first micro-chamber(12) embedded in the substrate (silicon) is accessible from the outside, and/or wherein the at least one of the first micro-channel (11) and/or at least one of the first micro-chamber(12) embedded in the substrate (silicon) have a height of 50-2000 µm, and/or wherein the at least one of the second micro-channel (21) and/or at least one of the second micro-chamber(22) embedded in the polymer have a height of 1-1000 µm, and/or wherein at least one of the first micro-channel (11) and microchamber (22) comprises at least one column made of polymer, which connects the top and the

bottom side of the channel, preferably at least one oriented column, preferably an array of c*d columns, and/or wherein the walls of the microchamber (12) and/or microchannel (11) are coated with an insulating layer, such as a polymer, and/or with a conductive layer, such as a metal, and/or wherein the device includes one microchannel (21) and/or wherein the microchamber (22) is not connected via a hole array (28) to the microchamber (12) and/or is not connected to a microchannel (16).

10. Device according to any of the preceding claims, wherein the polymer layers (20a,20b) are provided with openings, the openings providing access to at least one of a metal pad, an IC, a sensor, such as an optical sensor, and a heater.

11. Method for producing a device (100) according to any of the preceding claims, comprising the steps of

providing a Si-substrate (10), optionally comprising at least one sensor (90),

a1) depositing/growing a first dielectric layer (51a,b) on both sides of the substrate, and

b) patterning the dielectric layer on a bottom side;

c) depositing a first membrane polymer layer (61) on the unpatterned Si-side of the dielectric layer;

d) patterning the first membrane layer (61) using a lithography or electron beam machine;

e1) depositing a sacrificial layer (72) on the first membrane layer;

e2) patterning the sacrificial layer (72) using a lithography or electron beam machine; and etching the polymer layer with plasma etching/ dry etching;

f) depositing a second membrane layer (62) on the sacrificial layer (72);

g) dry etching the Silicon substrate (10) at the bottom side, therewith providing openings for channels (11)/chambers (12) in Si;

h) (wet) etching of the first dielectric layer (51b) from the bottom side providing openings for channels/chambers; and

i) (wet) etching of the sacrificial layer (72) thereby releasing channels (21)/chambers (22).

12. Method according to claim 11, wherein the first and second dielectric layers (51a,b,52) are made from a material independently selected from Si-dielectric materials, such as SiO_2, and Si_3N_4.

13. Method according to any of claims 11-12, wherein a thickness of the first (51a,b), second (52), and third (53) dielectric layer are each independently from 10-50000 nm.

14. Method according to any of claims 11-13, wherein a thickness of the first membrane layer (61) is from 50-30000 nm.

15. Method according to any of claims 11-14, wherein a thickness of the second membrane layer (62) is from 50-2000 µm.

16. Method according to any of claims 11-15, wherein the membrane layers (61,62) are each independently made from a material selected from a biopolymer, preferably a biocompatible polymer, such as polysiloxane, such as PDMS, polyimides, and parylene, and biodegradable polymers, such as Biorubber (PGS) and poly(1,8-octanediol-co-citrate) (POC), and combinations thereof.

17. Method according to any of claims 11-16, wherein the sacrificial layer (72) is a photo resist, such as an I-line photo resist, or a silicon oxide.

18. Method according to any of claims 11-17, wherein patterning is performed using an I-line lithographic machine.

19. Method according to any of claims 11-18, wherein at least one dielectric layer is formed by one of PECVD, and oxidation.

20. Method according to any of claims 11-19, wherein dry etch of silicon is performed using DRIE and/or wherein wet etching of silicon is performed using KOH.

21. Method according to any of claims 11-20, wherein at least one of the membrane layers is spun.

22. Method for producing a device (100) according to any of claims 1-10, comprising at least one step of
providing a substrate (10), optionally comprising at least one sensor (90), a microelectrode array (29), and at least two sets of microgrooves (27);

a1) depositing/growing a first dielectric layer (51a,b) on at least one side of the substrate, and

a2) patterning the dielectric layer on a top and/or bottom side;

b1) depositing a metal layer on the top side of the substrate;

b2) patterning the metal layer (81);

c1) depositing a first flexible and/or stretchable dielectric layer on the top side of the substrate;

c2) patterning the first flexible and or stretchable dielectric layer (52);

d1) depositing a conductive layer on the top side of the substrate;

d2) patterning the conductive layer (82);

e1) depositing a second flexible and/or stretchable dielectric layer;

e2) patterning the second flexible and/or stretchable dielectric layer (53);

f1) depositing a first sacrificial layer for a first set of micro-grooves;

f2) patterning the first sacrificial layer (71);

g) depositing a first membrane layer (61) on the second dielectric layer;

h) patterning the first membrane layer (61);

i) partially etching the first membrane layer (61) to define a second set of microgrooves;

l1) depositing a second sacrificial layer (72) on the first membrane layer,

l2) patterning the second sacrificial layer (72);

m) depositing a second membrane layer (62) on the sacrificial layer (71);

n) patterning the second membrane layer (62);

o) etching the substrate (10) at the bottom side, therewith providing openings for channels (11)/chambers (12) in the substrate;

p1) deposition of a conductive or isolating chamber coating (14);

p1) etching of the conductive or isolating chamber coating;

q) etching of the first dielectric layer (51b) from the bottom side providing openings for channels/chambers; and

r) etching of the sacrificial layer (72) thereby releasing channels (21)/chambers (22).

23. Method according to any of claims 11-22, wherein dimensions of the at least one first micro-channel (11) and/or at least one first micro-chamber(12) embedded in the substrate (silicon), the at least one input (16), the at least one second micro-channel (21) and/or at least one second micro-chamber(22) embedded in the polymer bottom layer, are each independently fully adaptable in a range of 50 nm-5 mm, and/or wherein the dimensions of the matrix of holes (28), the micro-features, are each independently fully adaptable in a range of 50 nm-20 µm.

24. Method according to any of claims 11-23, wherein the substrate layer comprises at least two alignment markers, and wherein during at least one method step the substrate (10) is aligned.

25. Use of a device according to any of claims 1-10, for at least one of a biological cell experiment, an organ on a chip experiment, an optical microscope experiment, growth and differentiation of primary cell experiment, mechanical and electrical stimulation of a cell, a stratified structure, simulation of a microenvironment in living tissue and/or organ, as Lab-on-Chip, as a microfluidics device, and as a micro-reactor.

26. Use of a device according to any of claims 1-10, wherein a wet/humid section and a dry section of the device are physically separated, wherein the dry section comprises electronics.

CONCLUSIES

1. Inrichting (100) voor micro-fluidica omvattende

(a) op polymeer gebaseerde micro-fluïdica (20), omvattende een 0,05-30 µm dunne polymere toplaag (20a) waarbij de polymeer toplaag bij voorkeur een matrix van gaten (28) daarin heeft,
een 50-2000 µm polymeer onderste laag (20b) in contact met de polymere toplaag, en omvattende ten minste één tweede microkanaal (21) en/of ten minste één tweede micro-kamer (22) ten minste gedeeltelijk ingebed in de polymere onderlaag,

(b) op substraat gebaseerde micro-fluïdica (10) in microfluidisch contact met de toplaag (20a) van de op polymeer gebaseerde micro-fluïdica waarbij de op silicium gebaseerde microfluïdica toegankelijk zijn en/of kan toegankelijk worden gemaakt voor het gebruik van het apparaat, omvattend
ten minste één eerste microkanaal (11) en/of ten minste één eerste micro-kamer (12) ten minste gedeeltelijk ingebed in het silicoon, en ten minste een ingang (16),
waarbij de ingang (16) in microfluïdisch contact is met het ten minste ene tweede microkanaal (21) en/of de ten minste ene tweede microkamer (22) ingebed in de polymere onderlaag, en
waarbij de polymeertoplaag (20a) het ten minste één eerste micro-kanaal (11) en/of ten minste ene eerste microkamer (12) ingebed in het substraat (silicium) afscheidt van ten minste één van de tweede micro-kanalen (21) en/of ten minste één van de tweede micro-kamer (22) ingebed in de polymere onderlaag, bij voorkeur ten minste gedeeltelijk door de matrix van gaten (28) daarin.

2. Inrichting volgens conclusie 1, verder omvattend ten minste één van een microchip, een ingebouwde sensor, en een uitgang (18).

3. Inrichting volgens een der voorgaande conclusies, waarbij het membraan rekbaar is met een treksterkte van > 1 [MPa] (ISO 527) en/of flexibel is met een elasticiteitsmodulus

<3 [GPa] (ISO 527), of waarbij het membraan stijf is met een Young's modulus van > 10 [GPa] (ISO 527).

4. Inrichting volgens een der voorgaande conclusies, waarbij het polymeer onafhankelijk gekozen is uit biocompatibele polymeren, zoals polysiloxanen, zoals polydimethylsiloxaan (PDMS), polyimides, polyurethaan, butylrubber, styreen-ethyleen-butyleen-styreen (SEBS), polypropyleen, polycarbonaat, polyester, polypropyleen, en biologisch afbreekbare poly meren, zoals Biorubber (PGS) en poly (1,8-octaandiol-co-citraat) (POC), en combinaties daarvan.

5. Inrichting volgens een der voorgaande conclusies, waarbij het membraan een reeks nxm openingen, waarbij een dichtheid van gaten 0,001-250/100 μm^2 is, en/of waarbij een gemiddelde gatgrootte 0,05-500 μm^2 is.

6. Inrichting volgens één der voorgaande conclusies, verder omvattende ingebed in de inrichting ten minste een van een sensor, een pomp, een micro-elektrode, een klep, een rekstrookje, een actuator, een verwarmingsinrichting, een koeler, een stimulator , een stroom sensor, een temperatuur sensor, een pH-sensor, een IC-circuit, een versterker, een actuator, een hete plaat, een micro-elektrode-array, een chemische stimulator, een optische stimulator, een ion sensor, een drukregelaar, en verdere microfluïdische elementen.

7. Inrichting volgens een der voorgaande conclusies, omvattend ingebed in de dunne polymere toplaag (20a) ten minste één elektrode (29), waarbij de elektrode bij voorkeur een toegankelijk gebied (29a) van 0,1-5000 μm^2 heeft.

8. Inrichting volgens één der voorgaande conclusies, waarbij de dunne polymere toplaag (20a) omvat op tenminste een zijde daarvan, tenminste één micro-kenmerk, zoals een inkeping, een groef, een topografische structuur, bij voorkeur tenminste één georiënteerde microgroef, bij voorkeur een reeks van x * y georiënteerd microgroeven, waarbij een dichtheid van microgroeven 1-25/100 μm^2 is, en/of waarbij een gemiddeld groefoppervlak 0,1-10^6 μm^2 is, en/of waarbij het ten minste een microkenmerk is uitgelijnd ten opzichte van de inrichting.

9. Inrichting volgens een der voorgaande conclusies, waarbij ten minste één van het eerste micro-kanaal (11) en/of ten minste één van de eerste micro-kamer (12) ingebed in het

substraat (silicium) van buitenaf toegankelijk is en/of waarbij ten minste een van het eerste micro-kanaal (11) en/of ten minste één van de eerste micro-kamer (12) ingebed in het substraat (silicium) een hoogte van 50-2000 µm heeft, en/of waarbij ten minste één van het tweede microkanaal (21) en/of ten minste één van de tweede micro-kamer (22) ingebed in het polymeer een hoogte van 1-1000 µm heeft, en/of waarbij ten minste één van het eerste micro -kanaal (11) en microkamer (22) ten minste één kolom uit kunststof omvat, die de boven- en onderzijde van het kanaal verbindt, bij voorkeur tenminste één georiënteerde kolom, bij voorkeur een reeks van c * d kolommen, en/of waarbij de wanden van de microkamer (12) en/of microkanaal (11) zijn bedekt met een isolerende laag, zoals een polymeer, en/of met een geleidende laag, zoals een metaal, en/of waarbij de inrichting één microkanaal (21) omvat en/of waarbij de microkamer (22) die niet is verbonden via een gatmatrix (28) met de microkamer (12) en/of niet is verbonden met een microkanaal (16).

10. Inrichting volgens een der voorgaande conclusies, waarbij de polymeerlagen (20a, 20b) zijn voorzien van openingen, waarbij de openingen toegang tot ten minste één van een metalen blok, een IC, een sensor, bijvoorbeeld een optische sensor, en een verwarmer, verschaffen.

11. Werkwijze voor het vervaardigen van een inrichting (100) volgens één der voorgaande conclusies, omvattende de stappen van

het verschaffen van een Si-substraat (10), eventueel omvattende ten minste één sensor (90),

a1) het afzetten/groeien van een eerste diëlektrische laag (51a, b) aan beide zijden van het substraat, en

b) het patroonvormen van de diëlektrische laag op een onderkant;

c) het afzetten van een eerste membraan polymeerlaag (61) op de niet-gepatroneerde Si-zijde van de diëlektrische laag;

d) het patroonvormen van de eerste membraan laag (61) met een lithografie- of elektronenbundel machine;

e1) het afzetten van een opofferingslaag (72) op de eerste membraan laag;

e2) het patroonvormen van de opofferingslaag (72) met behulp van een lithografie- of elektronenbundel machine; en het etsen van de polymeerlaag met plasma etsen/droog etsen;

f) het afzetten van een tweede membraan laag (62) op de opofferingslaag (72);

g) het droog etsen van het siliciumsubstraat (10) aan de onderzijde, daarmee openingen verschaffend voor de kanalen (11)/kamers (12) in Si;

h) het (nat) etsen van de eerste diëlektrische laag (51b) vanaf de onderzijde daarmee openingen verschaffend voor kanalen/kamers; en

i) het (nat) etsen van de opofferingslaag (72), waardoor kanalen (21)/kamers (22) vrij komen.

12. Werkwijze volgens conclusie 11, waarbij de eerste en tweede diëlektrische lagen (51a, b, 52) gemaakt zijn van een materiaal onafhankelijk gekozen uit Si-diëlektrische materialen, zoals SiO_2, en Si_3N_4.

13. Werkwijze volgens één van de conclusies 11-12, waarbij een dikte van de eerste (51a, b), tweede (52) en derde (53) diëlektrische lagen elk onafhankelijk 10-50000 nm zijn.

14. Werkwijze volgens één van de conclusies 11-13, waarbij een dikte van de eerste membraanlaag (61) is 50-30000 nm.

15. Werkwijze volgens één van de conclusies 11-14, waarbij een dikte van de tweede membraanlaag (62) is 50-2000 µm.

16. Werkwijze volgens één van de conclusies 11-15, waarbij de membraanlagen (61,62) elk onafhankelijk gemaakt zijn van een materiaal gekozen uit een biopolymeer, bij voorkeur een biocompatibel polymeer, zoals polysiloxaan, zoals PDMS, polyimides, en paryleen, en biologisch afbreekbare polymeren, zoals Biorubber (PGS) en poly (1,8-octaandiol-co-citraat) (POC), en combinaties daarvan.

17. Werkwijze volgens één van de conclusies 11-16, waarbij de opofferings laag (72) een fotoresist, zoals een I-lijn fotoresist, of een siliciumoxide is.

18. Werkwijze volgens één van de conclusies 11-17, waarbij patroonvormen wordt uitgevoerd met een I-lijn lithografische machine.

19. Werkwijze volgens één van de conclusies 11-18, waarbij ten minste één diëlektrische laag wordt gevormd door één van PECVD en oxidatie.

20. Werkwijze volgens één van de conclusies 11-19, waarbij droge etsen van silicium wordt uitgevoerd met DRIE en/of waarbij nat etsen van silicium wordt uitgevoerd met KOH.

21. Werkwijze volgens één van de conclusies 11-20, waarbij ten minste één van de membraanlagen wordt gesponnen.

22. Werkwijze voor het vervaardigen van een inrichting (100) volgens één van de conclusies 1-10, omvattende ten minste één stap van

het verschaffen van een substraat (10), eventueel omvattende ten minste één sensor (90), een micro-elektrode array (29), en ten minste twee sets van microgroeven (27);

a1) het afzetten/groeien van een eerste diëlektrische laag (51a, b) op ten minste één zijde van het substraat en

a2) patroonvorming van de diëlektrische laag op een boven- en/of onderzijde;

b1) het afzetten van een metaallaag op de bovenzijde van het substraat;

b2) het patroonvormen van de metaallaag (81);

c1) afzetten van een eerste flexibele en/of rekbare diëlektrische laag op de bovenzijde van het substraat;

c2) het patroonvormen van de eerste flexibele en/of rekbare diëlektrische laag (52);

d1) het afzetten van een geleidende laag op de bovenzijde van het substraat;

d2) het patroonvormen van de geleidende laag (82);

e1) het afzetten van een tweede flexibele en/of rekbare dielektrische laag;

e2) het patroonvorming van de tweede flexibele en/of rekbare diëlektrische laag (53);

f1) het afzetten van een eerste opofferingslaag voor een eerste reeks micro-groeven;

f2) het patroonvormen van de eerste opofferingslaag (71);

g) het afzetten van een eerste membraanlaag (61) op de tweede diëlektrische laag;

h) het patroonvormen van de eerste membraanlaag (61);

i) het gedeeltelijk etsen van de eerste membraanlaag (61) om een tweede set microgroeven te definiëren;

l1) het afzetten van een tweede opofferingslaag (72) op de eerste membraanlaag,

l2) het patroonvormen van de tweede opofferingslaag (72);

m) het afzetten van een tweede membraan laag (62) op de opofferingslaag (71);

n) het patroonvormen van de tweede membraanlaag (62);

o) het etsen van het substraat (10) aan de onderzijde, daarmee openingen verschaffend voor de kanalen (11)/kamers (12) in het substraat;

p1) het afzetten van een geleidende of isolerende kamerbekleding (14);

p1) het etsen van de geleidende of isolerende kamerbekleding;

q) het etsen van de eerste diëlektrische laag (51b) van de onderzijde voor het verschaffen van openingen voor kanalen/kamers; en

r) het etsen van de opofferingslaag (72), waardoor kanalen (21/kamers (22) vrij komen.

23. Werkwijze volgens één van de conclusies 11-22, waarbij afmetingen van het ten minste ene eerste microkanaal (11) en/of ten minste één eerste micro-kamer (12) ingebed in het substraat (silicium), de ten minste ene ingang (16), het ten minste ene tweede microkanaal (21) en/of ten minste één tweede micro-kamer (22) ingebed in de polymere onderlaag, elk onafhankelijk volledig aanpasbaar in een bereik van 50 nm-5 mm, en/of waarbij de afmetingen van de matrix van gaten (28), de micro-elementen, elk onafhankelijk volledig aanpasbaar in een bereik van 50 nm-20 µm.

24. Werkwijze volgens één van de conclusies 11-23, waarbij de substraatlaag tenminste twee uitlijnmarkeringen omvat, en waarbij gedurende ten minste één werkwijzestap het substraat (10) wordt uitgelijnd.

25. Gebruik van een inrichting volgens één van de conclusies 1-10, voor ten minste één van een biologisch celexperiment, een orgaan op een chip experiment, een optische microscoop experiment, groei en differentiatie van primaire cel experiment, mechanische en elektrische stimulatie van een cel, een gelaagde structuur, simulatie van een micro-omgeving in

levend weefsel en/of orgaan, zoals Lab-on-Chip, als microfluidische apparaat, en als een microreactor.

26. Gebruik van een inrichting volgens één der conclusies 1-10, waarbij een nat/vochtig gedeelte en een droog gedeelte van de inrichting fysiek gescheiden zijn, waarbij het droge gedeelte elektronica omvat.

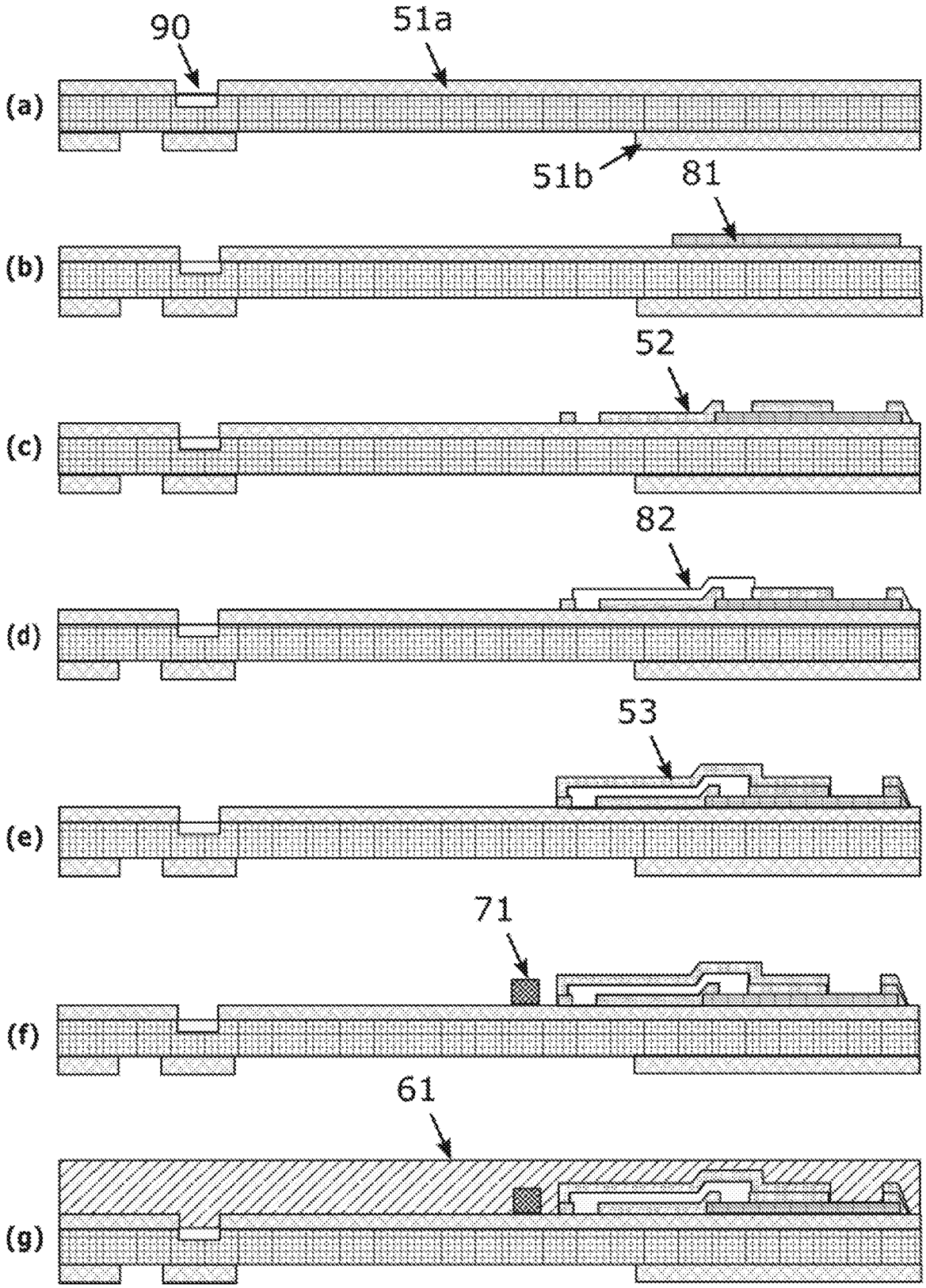


Fig. 1a-g

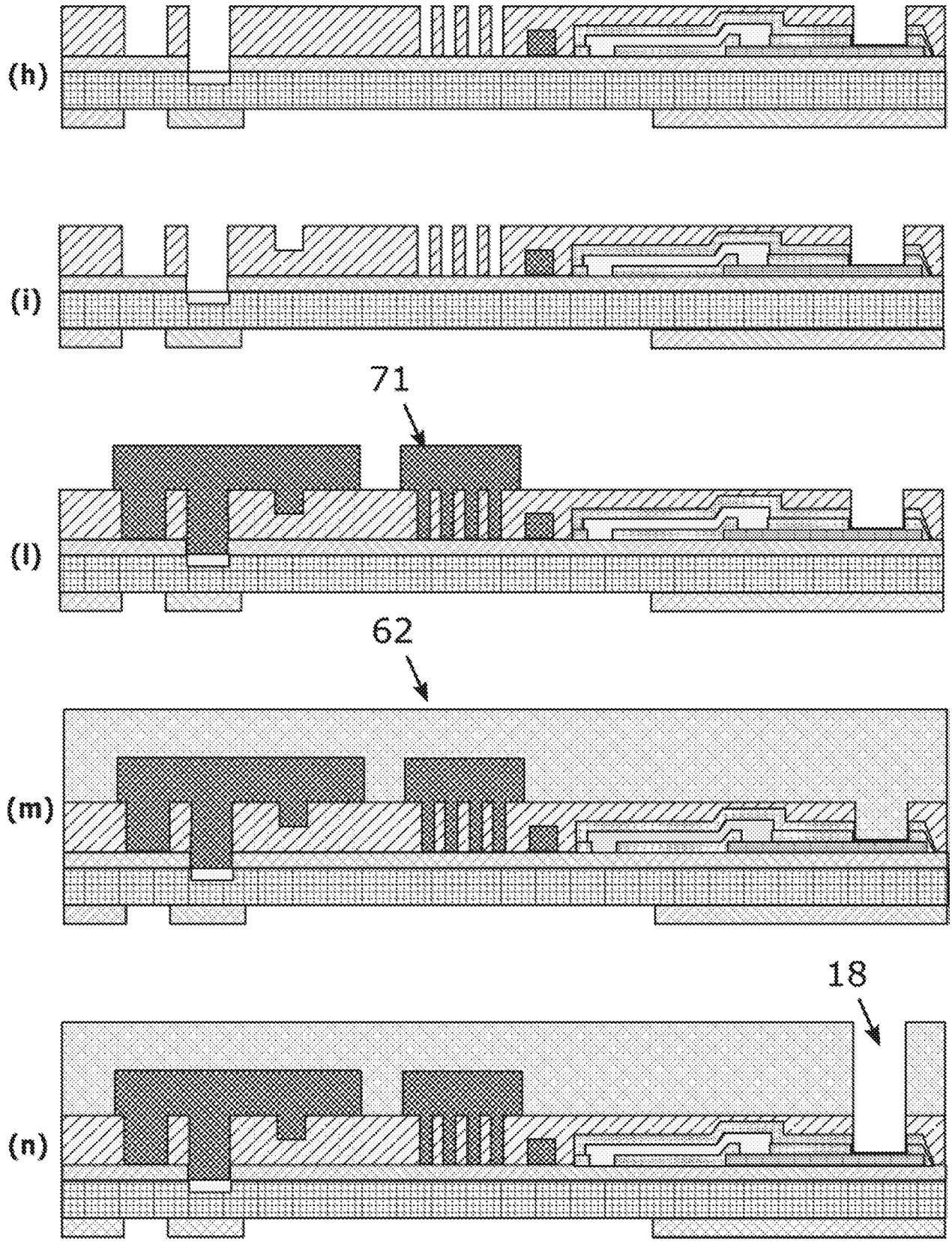


Fig. 1h-n

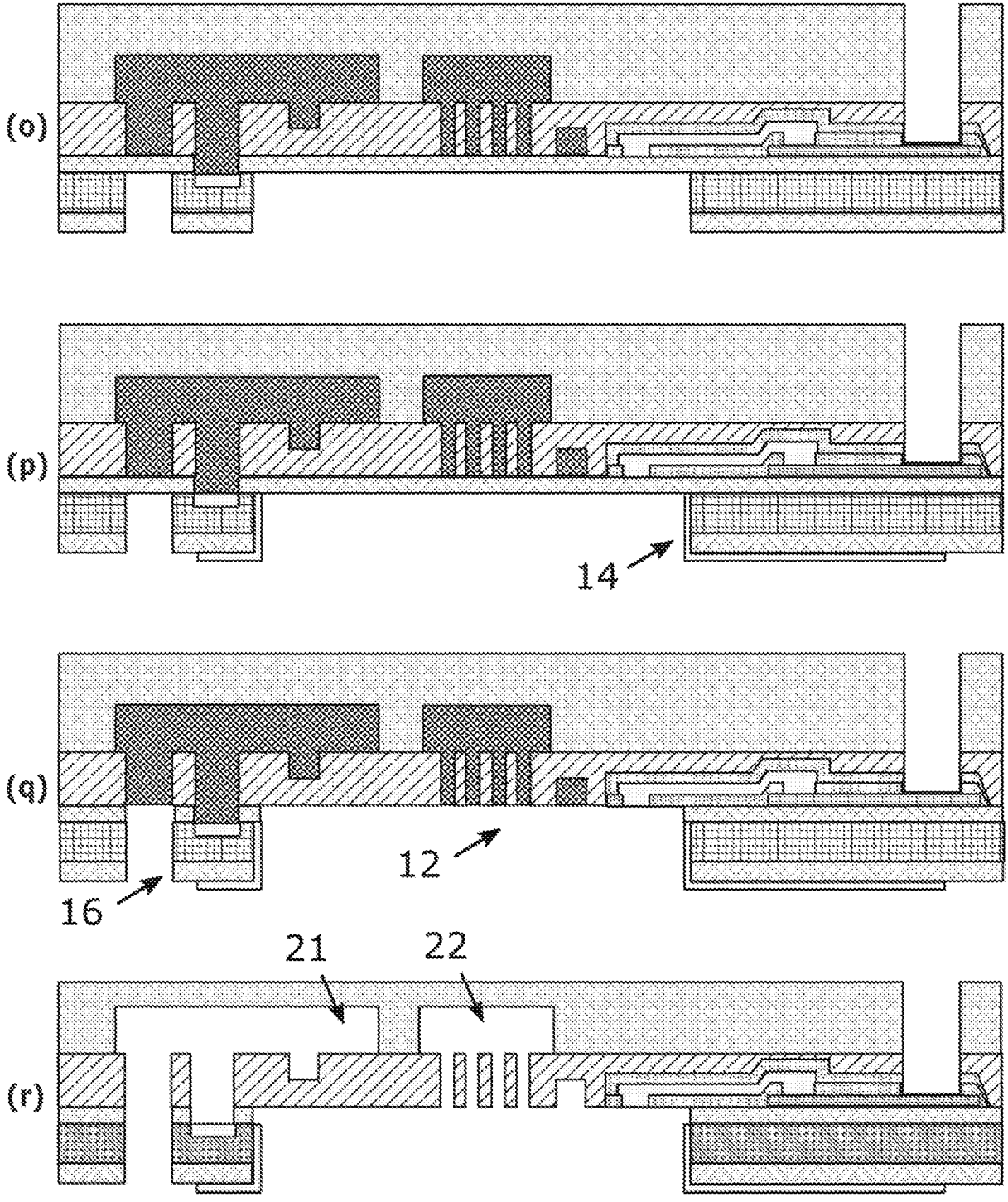


Fig. 1o-r

Fig. 2a

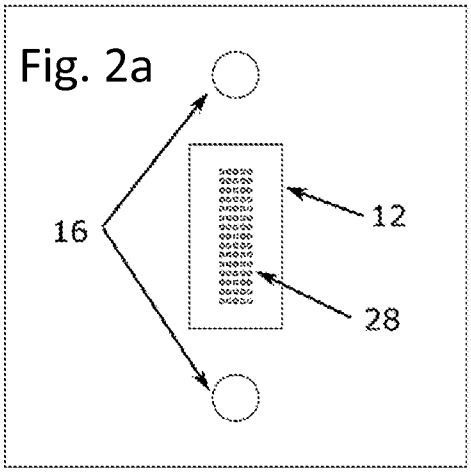


Fig. 2b

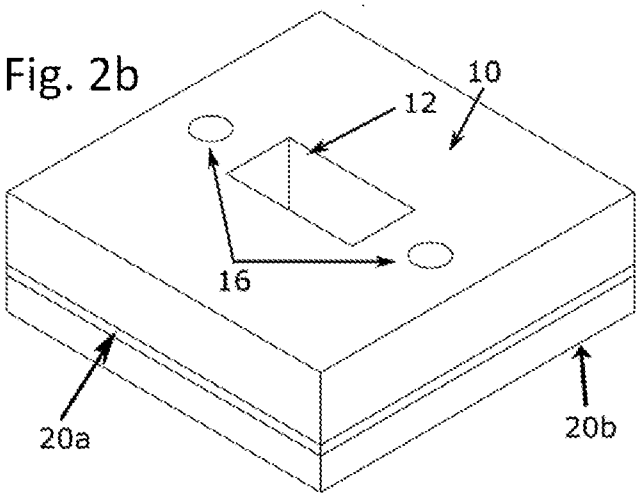


Fig. 2c

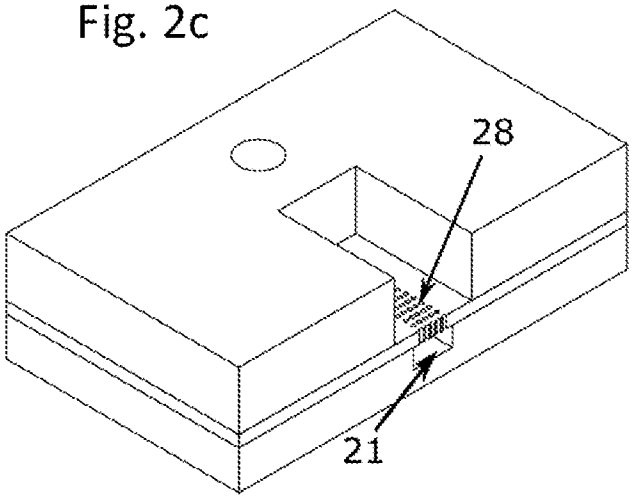


Fig. 2d

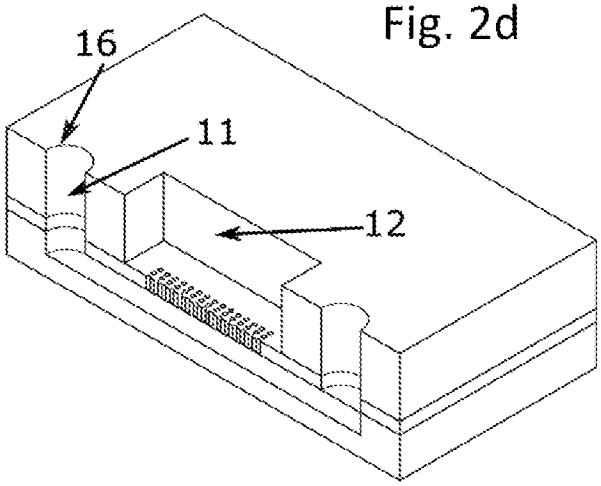


Fig. 3a

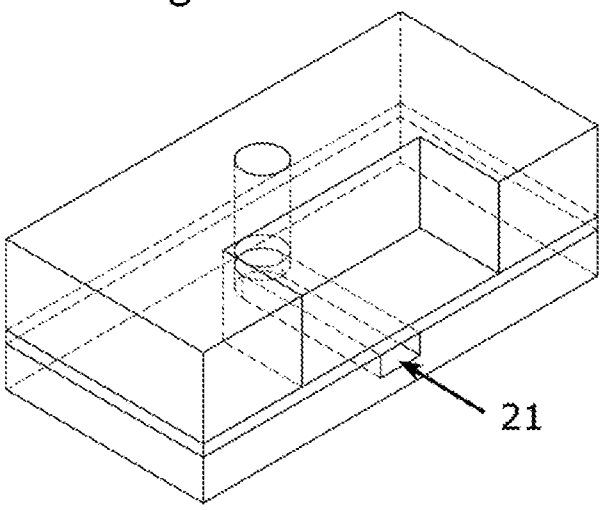


Fig. 3b

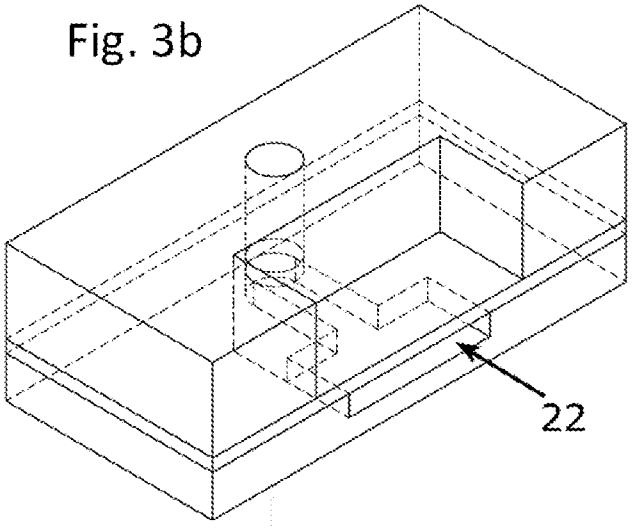


Fig. 4

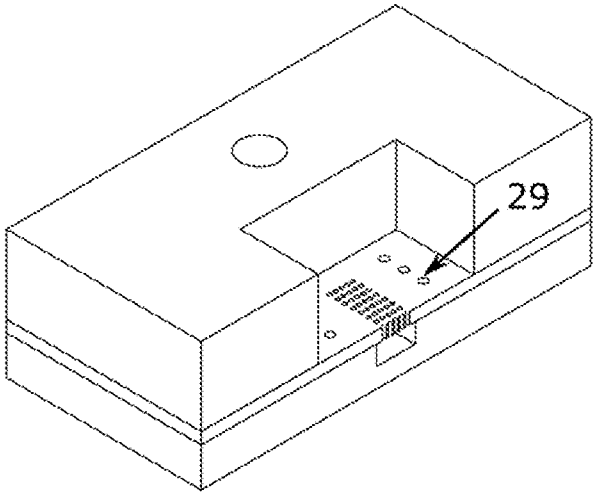


Fig. 5

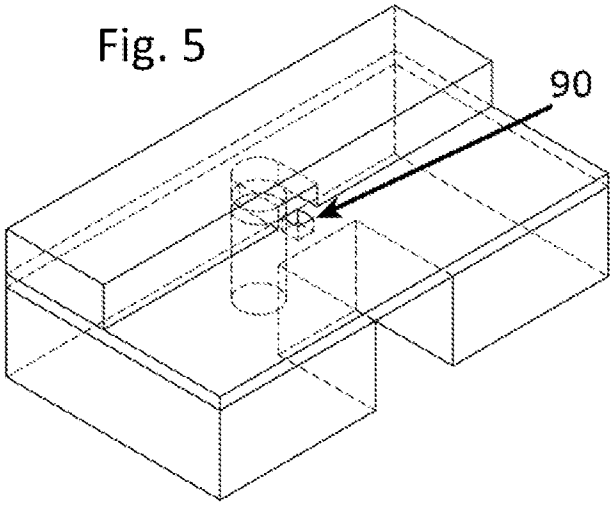


Fig. 6

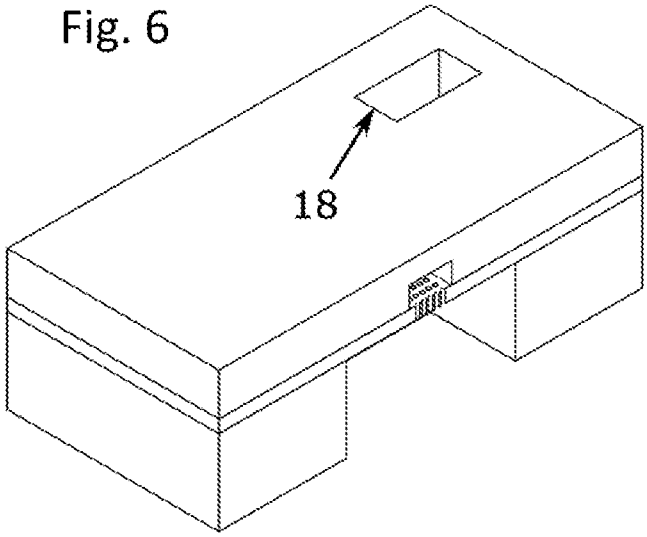


Fig. 7

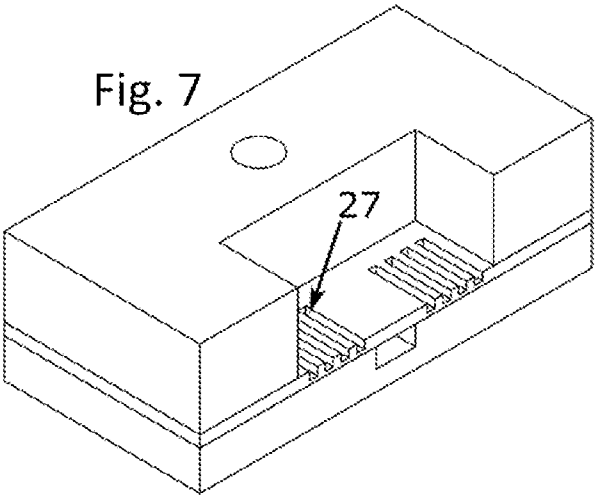


Fig. 8

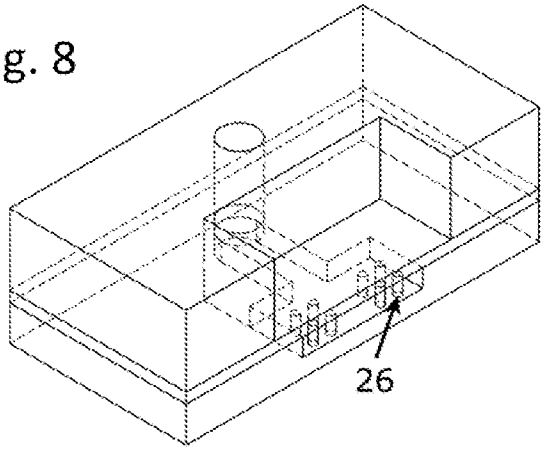


Fig. 9

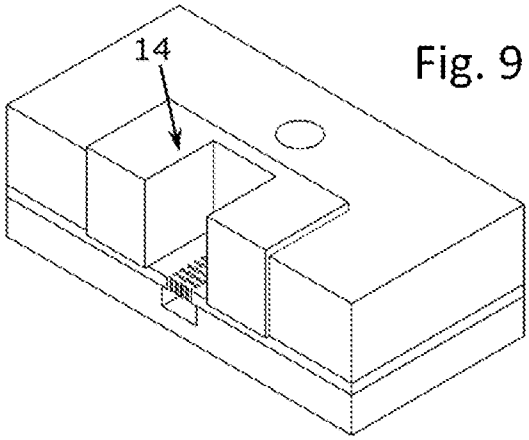


Fig. 10a

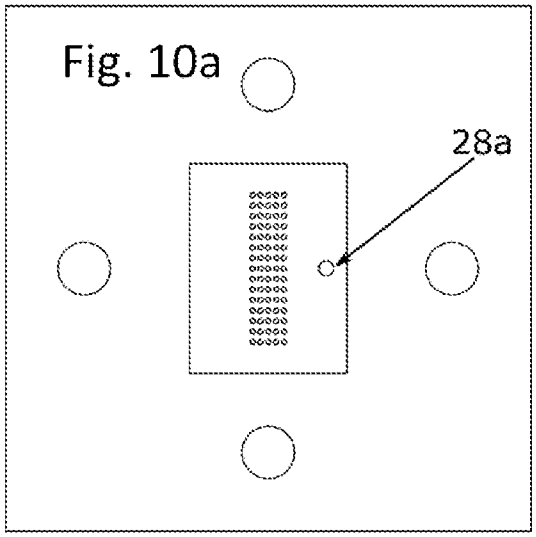


Fig. 10b

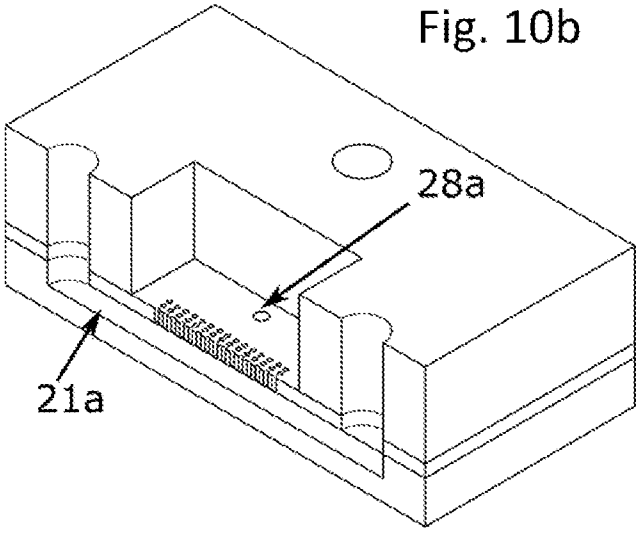


Fig. 10c

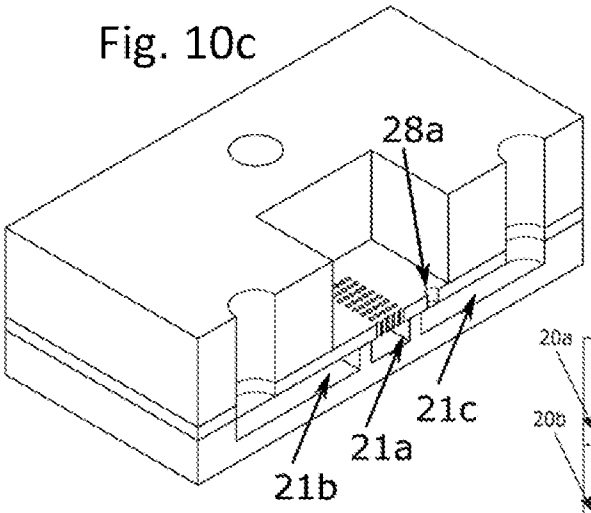
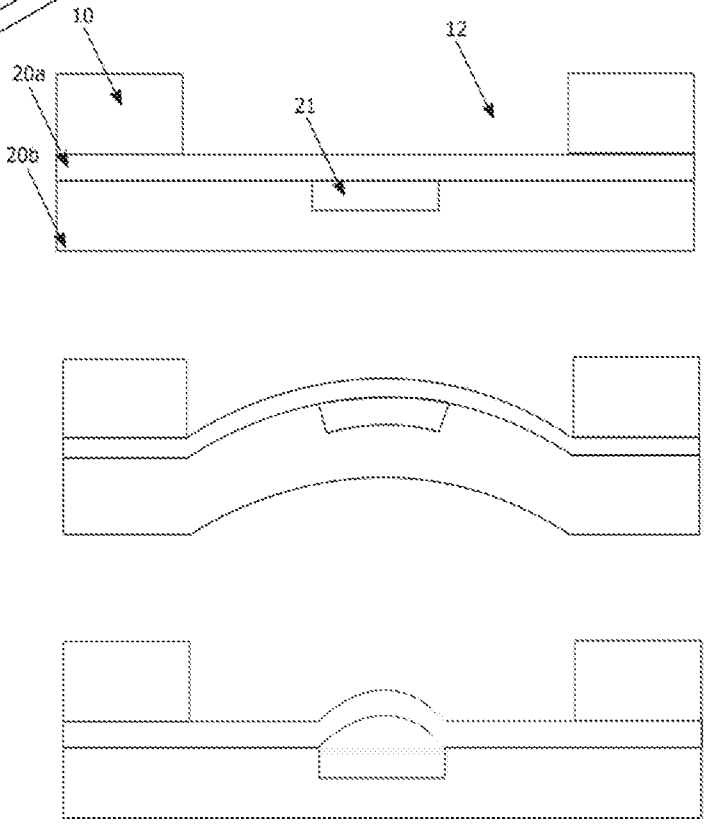


Fig. 11



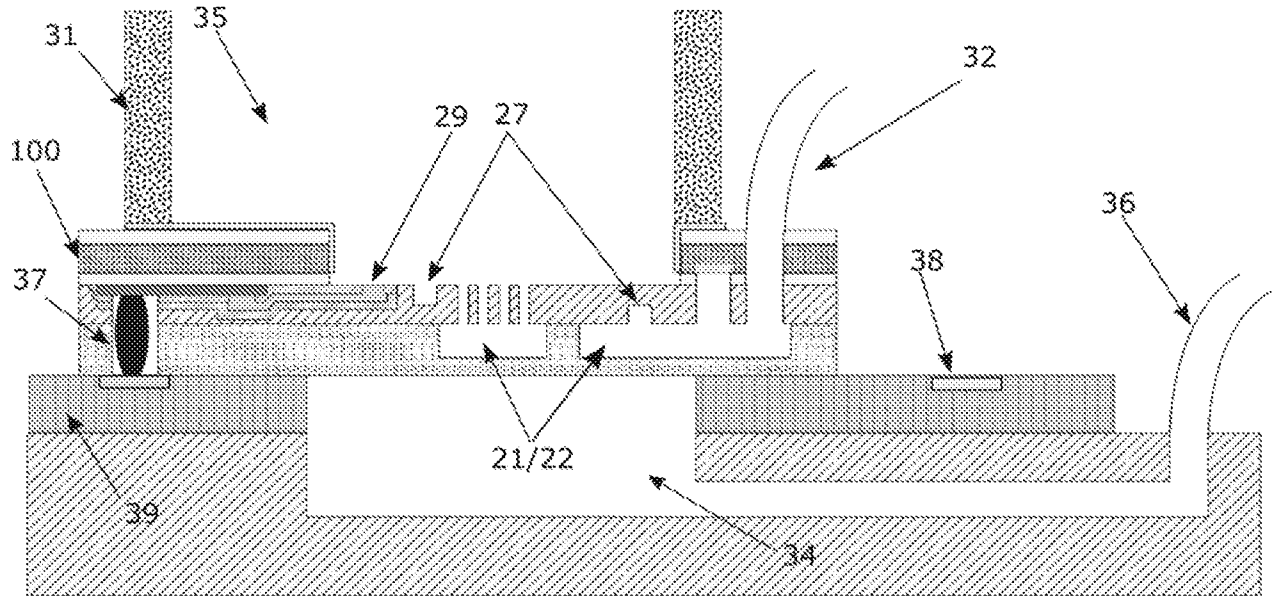


Fig. 12

ABSTRACT

The present invention is in the field of microfluidic devices produced with silicon technology wherein at least one 3D microenvironment is present, a method of producing said device using silicon based technology, and a use of said device in various applications, typically a biological cell experiment, such as a cell or organ on a chip experiment, and use of the device as a microreactor.

SAMENWERKINGSVERDRAG (PCT)

RAPPORT BETREFFENDE NIEUWHEIDSONDERZOEK VAN INTERNATIONAAL TYPE

IDENTIFICATIE VAN DE NATIONALE AANVRAGE	KENMERK VAN DE AANVRAGER OF VAN DE GEMACHTIGDE
	016935 NL-PD
Nederlands aanvraag nr. **2017227**	Indieningsdatum **25-07-2016**
	Ingeroepen voorrangsdatum

Aanvrager (Naam)

Technische Universiteit Delft

Datum van het verzoek voor een onderzoek van internationaal type	Door de instantie voor Internationaal Onderzoek aan het verzoek voor een onderzoek van internationaal type toegekend nr.
24-09-2016	**SN67424**

I. CLASSIFICATIE VAN HET ONDERWERP (bij toepassing van verschillende classificaties, alle classificatiesymbolen opgeven)

Volgens de internationale classificatie (IPC)

B01L3/00

II. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimumdocumentatie

Classificatiesysteem	Classificatiesymbolen
IPC	**B01L;C12M**

Onderzochte andere documentatie dan de minimum documentatie, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

III.	☐ **GEEN ONDERZOEK MOGELIJK VOOR BEPAALDE CONCLUSIES**	(opmerkingen op aanvullingsblad)
IV.	☐ **GEBREK AAN EENHEID VAN UITVINDING**	(opmerkingen op aanvullingsblad)

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2017227

A. CLASSIFICATIE VAN HET ONDERWERP
INV. B01L3/00
ADD.

Volgens de Internationale Classificatie van octrooien (IPC) of zowel volgens de nationale classificatie als volgens de IPC.

B. ONDERZOCHTE GEBIEDEN VAN DE TECHNIEK

Onderzochte minimum documentatie (classificatie gevolgd door classificatiesymbolen)
B01L C12M

Onderzochte andere documentatie dan de minimum documentatie, voor dergelijke documenten, voor zover dergelijke documenten in de onderzochte gebieden zijn opgenomen

Tijdens het onderzoek geraadpleegde elektronische gegevensbestanden (naam van de gegevensbestanden en, waar uitvoerbaar, gebruikte trefwoorden)
EPO-Internal, WPI Data

C. VAN BELANG GEACHTE DOCUMENTEN

Categorie *	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
X	WO 2015/138034 A2 (HARVARD COLLEGE [US]) 17 september 2015 (2015-09-17) * alineas [0022], [0088], [0091], [0093], [0094], [0097] - [0101], [0106], [0109], [0115] * * figuren 1, 2A-2D * -----	1-10
X	NIKOLAS GAIO ET AL: "Cytostretch, an Organ-on-Chip Platform", MICROMACHINES, deel 7, nr. 7, 14 juli 2016 (2016-07-14), bladzijde 120, XP055366704, DOI: 10.3390/mi7070120	11-24
A	* bladzijde 2 * * bladzijde 4 * * bladzijde 6 - bladzijde 7 * * figuren 1, 3, 4, 8 * -----	1-10,25

-/--

[X] Verdere documenten worden vermeld in het vervolg van vak C.

[X] Leden van dezelfde octrooifamilie zijn vermeld in een bijlage

* Speciale categorieën van aangehaalde documenten

"A" niet tot de categorie X of Y behorende literatuur die de stand van de techniek beschrijft

"D" in de octrooiaanvrage vermeld

"E" eerdere octrooi(aanvrage), gepubliceerd op of na de indieningsdatum, waarin dezelfde uitvinding wordt beschreven

"L" om andere redenen vermelde literatuur

"O" niet-schriftelijke stand van de techniek

"P" tussen de voorrangsdatum en de indieningsdatum gepubliceerde literatuur

"T" na de indieningsdatum of de voorrangsdatum gepubliceerde literatuur die niet bezwarend is voor de octrooiaanvrage, maar wordt vermeld ter verheldering van de theorie of het principe dat ten grondslag ligt aan de uitvinding

"X" de conclusie wordt als niet nieuw of niet inventief beschouwd ten opzichte van deze literatuur

"Y" de conclusie wordt als niet inventief beschouwd ten opzichte van de combinatie van deze literatuur met andere geciteerde literatuur van dezelfde categorie, waarbij de combinatie voor de vakman voor de hand liggend wordt geacht

"&" lid van dezelfde octrooifamilie of overeenkomstige octrooipublicatie

Datum waarop het onderzoek naar de stand van de techniek van internationaal type werd voltooid	Verzenddatum van het rapport van het onderzoek naar de stand van de techniek van internationaal type
24 april 2017	

Naam en adres van de instantie	De bevoegde ambtenaar
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bischoff, Laura

2

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2017227

C.(Vervolg). VAN BELANG GEACHTE DOCUMENTEN

Categorie *	Geciteerde documenten, eventueel met aanduiding van speciaal van belang zijnde passages	Van belang voor conclusie nr.
A	@ Chemcareers ET AL: "Characterization of a microfluidic in vitro model of the blood-brain barrier (mBBB)", , 1 maart 2012 (2012-03-01), XP055363984, Gevonden op het Internet: URL:http://pubs.rsc.org/en/content/articlepdf/2012/lc/c2lc40094d [gevonden op 2017-04-11] * het gehele document *	1-26
A	KYUNG-JIN JANG ET AL: "A multi-layer microfluidic device for efficient culture and analysis of renal tubular cells", LAB ON A CHIP, deel 10, nr. 1, 26 augustus 2009 (2009-08-26), bladzijden 36-42, XP055363987, ISSN: 1473-0197, DOI: 10.1039/B907515A * het gehele document *	1-26
A	US 2004/045891 A1 (GILBERT JOHN RICHARD [US] ET AL) 11 maart 2004 (2004-03-11) * alineas [0039], [0042], [0043], [0045], [0046], [0049], [0056] * * figuren 2, 3 *	1-10,25, 26
A	US 2009/131858 A1 (FISSELL WILLIAM H [US] ET AL) 21 mei 2009 (2009-05-21) * het gehele document *	1-26
A	US 2006/154361 A1 (WIKSWO JOHN P [US] ET AL) 13 juli 2006 (2006-07-13) * het gehele document *	1-10

2

ONDERZOEKSRAPPORT BETREFFENDE HET RESULTAAT VAN HET ONDERZOEK NAAR DE STAND VAN DE TECHNIEK VAN HET INTERNATIONALE TYPE

Informatie over leden van dezelfde octrooifamilie

Nummer van het verzoek om een onderzoek naar de stand van de techniek
NL 2017227

In het rapport genoemd octrooigeschrift	Datum van publicatie	Overeenkomend(e) geschrift(en)	Datum van publicatie
WO 2015138034 A2	17-09-2015	AU 2014386209 A1	21-07-2016
		CA 2934662 A1	17-09-2015
		CN 106459898 A	22-02-2017
		EP 3083940 A2	26-10-2016
		GB 2538012 A	02-11-2016
		JP 2017504320 A	09-02-2017
		US 2016313306 A1	27-10-2016
		WO 2015138034 A2	17-09-2015
US 2004045891 A1	11-03-2004	AU 2003270553 A1	29-03-2004
		BR 0314145 A	12-07-2005
		CA 2498510 A1	18-03-2004
		EP 1545752 A2	29-06-2005
		IL 167305 A	20-07-2009
		JP 2005537923 A	15-12-2005
		KR 20050037603 A	22-04-2005
		SG 160202 A1	29-04-2010
		US 2004045891 A1	11-03-2004
		US 2005145497 A1	07-07-2005
		WO 2004022983 A2	18-03-2004
US 2009131858 A1	21-05-2009	EP 2125171 A1	02-12-2009
		US 2009131858 A1	21-05-2009
		US 2016332119 A1	17-11-2016
		WO 2008086477 A1	17-07-2008
US 2006154361 A1	13-07-2006	GEEN	

WRITTEN OPINION

File No.	Filing date *(day/month/year)*	Priority date *(day/month/year)*	Application No.
SN67424	25.07.2016		NL2017227

International Patent Classification (IPC)
INV. B01L3/00

Applicant
Technische Universiteit Delft

This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☐ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☒ Box No. VII Certain defects in the application
- ☒ Box No. VIII Certain observations on the application

Examiner
Bischoff, Laura

Application number

WRITTEN OPINION

NL2017227

Box No. I Basis of this opinion

1. This opinion has been established on the basis of the latest set of claims filed before the start of the search.
2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ on paper

   ☐ in electronic form

   c. time of filing/furnishing:

   ☐ contained in the application as filed.

   ☐ filed together with the application in electronic form.

   ☐ furnished subsequently for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
4. Additional comments:

Box No. V Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty	Yes:	Claims	3, 5, 10-26
	No:	Claims	1, 2, 4, 6-9
Inventive step	Yes:	Claims	25, 26
	No:	Claims	1-24
Industrial applicability	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

see separate sheet

WRITTEN OPINION

Box No. VII Certain defects in the application

see separate sheet

Box No. VIII Certain observations on the application

see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1 Reference is made to the following documents:

D1 WO 2015/138034 A2 (HARVARD COLLEGE [US]) 17 september 2015 (2015-09-17)

D2 NIKOLAS GAIO ET AL: "Cytostretch, an Organ-on-Chip Platform", MICROMACHINES,
deel 7, nr. 7, 14 juli 2016 (2016-07-14), bladzijde 120, XP055366704, DOI: 10.3390/mi7070120

2 **Novelty**

The present application does not meet the criteria of patentability, because the subject-matter of claims 1, 2, 7 and 25 is not new.

2.1 The claims of the present application contain so many options, and they are drafted in such a way that the claims as a whole are not in compliance with the provisions of clarity and conciseness, as it is particularly burdensome for a skilled person to establish the subject-matter for which protection is sought. The non-compliance with the substantive provisions is to such an extent, that the search was performed taking into consideration the non-compliance in determining the extent of the search.

The search was based on the subject-matter that, as far as can be understood, could reasonably be expected to be claimed later in the procedure, and the corresponding claims.

2.2 Document D1 discloses a micro-fluidic device (102, 200) comprising

(a) polymer based microfluidics (20), comprising

a 0.05-30 µm thin polymer top layer (208) the polymer top layer preferably having a matrix of holes therein,

a 50-2000 µm polymer bottom layer (206) in contact with the polymer top layer, and comprising at least one second micro-channel (225, 227) and at least one second micro-chamber (250B) at least partly embedded in the polymer bottom layer (see paragraphs [0091], [0094], [0098], [0101], [0106], [0115] and figures 2A-2D),

(b) substrate based microfluidics (204) in microfluidic contact with the top layer of the polymer based microfluidics wherein the silicon based microfluidics are accessible and/or can be made accessible for use of the device, comprising

at least one first micro-channel (225) and at least one first micro-chamber (250A) at least partly embedded in the substrate (silicon), and

at least one input (210, 211), the input being in microfluidic contact with the at least one second micro-channel and/or at least one second micro-chamber embedded in the polymer bottom layer (see paragraphs [0091], [0093], [0097], [0099], [0101], [0106], [0109] and figures 2A-2D), and

the polymer top layer separating at least one of the first micro-channel and/or at least one of the first micro-chamber embedded in the substrate (silicon) from at least one of the second micro-channel and/or at least one of the second micro-chamber embedded in the polymer bottom layer preferably at least partly by the matrix of holes therein (see paragraph [0115] and figures 2A-2D).

Therefore, the subject-matter of independent claim 1 is not novel.

2.3 The additional feature of dependent claim 2 is also anticipated by D1, which discloses that the device further comprises at least one of a microchip, an integrated sensor (120), and an output (see paragraphs [0088], [0100] and figure 1) .

Thus, the subject-matter of claim 2 is also not novel.

2.4 D1 also discloses that the device comprises embedded in the thin polymer top layer at least one electrode (102) (see paragraph [0088] and figure 1).

The subject-matter of dependent claim 7 can therefore also not be considered as novel.

2.5 The features of claim 25 are also anticipated by D1, since it discloses that the device can be used for, for at least one of a biological cell experiment, an organ on a chip experiment, an optical microscope experiment, growth and differentiation of primary cell experiment, mechanical and electrical stimulation of a cell, a stratified structure, simulation of a microenvironment in living tissue and/or organ, as Lab-on-Chip, as a microfluidics device, and as a micro-reactor (see paragraph [0022]).

Thus, the subject-matter of independent claim 25 is also not novel.

3 **Inventive step**

The present application does not meet the criteria of patentability, because the subject-matter of claims 11 and 22 does not involve an inventive step.

3.1 Document D2 is regarded as being the prior art closest to the subject-matter of claim 11, and shows a method for producing a device (see page 4), comprising the steps of providing a Si-substrate (see figure 3a),

a1) depositing/growing a first dielectric layer (SiO_2) on both sides of the substrate (see figure 3b), and

b) patterning the dielectric layer on a bottom side (see figures 3c);

c) depositing a first membrane polymer layer on the unpatterned Si-side of the dielectric layer (see figure 3d);

d) patterning the first membrane layer using a lithography machine (see figures 4a-4d);

g) dry etching the Silicon substrate at the bottom side, therewith providing openings for channels/chambers in Si (see figure 4e);

The subject-matter of claim 11 therefore differs from this known in D2 in that there is no deposition and patterning of a sacrificial layer on the first membrane layer, allowing deposition of a second membrane layer (steps e1, e2, f, h, i).

The subject-matter of claim 11 is therefore novel.

The problem to be solved by the present invention may therefore be regarded as providing the device with a second polymer layer containing a second microchannel/chamber.

The solution proposed in claim 11 of the present application cannot be considered as involving an inventive step. The device of D2, i.e. a PDMS membrane fabricated on a silicon chip, is said to be attached to a plastic cylinder (see page 2 - last paragraph and figures 1a-1c). Adding a second polymer layer containing a second microchamber is therefore already known from D2.

In view of the paragraph above, the skilled person would therefore regard it a normal procedure to combine all the features set out in claim 11.

3.2 Document D2 also describes a method for producing a device (see pages 4, 6, 7), comprising at least one step of providing a substrate (see figure 3a),

a1) depositing/growing a first dielectric layer (SiO_2) on at least one side of the substrate (see figure 3b), and

a2) patterning the dielectric layer on a top and/or bottom side (see figures 3c);

b1) depositing a metal layer on the top side of the substrate (see figure 8b);

b2) patterning the metal layer (see figure 8b);

c1) depositing a first flexible and/or stretchable dielectric layer on the top side of the substrate (see figure 8c);

c2) patterning the first flexible and or stretchable dielectric layer (see figure 8c);

d1) depositing a conductive layer on the top side of the substrate (see figure 8d);

d2) patterning the conductive layer (see figure 8d);

e1) depositing a second flexible and/or stretchable dielectric layer (see figure 8e);

e2) patterning the second flexible and/or stretchable dielectric layer (see figure 8e);

g) depositing a first membrane layer on the second dielectric layer (see figure 3d);

h) patterning the first membrane layer (see figures 4a-4d);

o) etching the substrate at the bottom side, therewith providing openings for channels/chambers in the substrate (see figure 4e);

The subject-matter of claim 22 therefore differs from this known in D2 in that there is no deposition and patterning of a sacrificial layer on the first membrane layer, allowing deposition of a second membrane layer.

The subject-matter of claim 22 is therefore novel.

The problem to be solved by the present invention may therefore be regarded as providing the device with a second polymer layer containing a second microchannel/chamber.

The solution proposed in claim 22 of the present application cannot be considered as involving an inventive step. The device of D2, i.e. a PDMS membrane fabricated on a silicon chip, is said to be attached to a plastic cylinder (see page 2 - last paragraph and figures 1a-1c). Adding a second polymer layer containing a second microchamber is therefore already known from D2.

In view of the paragraph above, the skilled person would therefore regard it a normal procedure to combine all the features set out in claim 22.

3.3 Dependent claims 3-6, 8-10, 12-21, 23-24 and 26 do not appear to contain any additional features which, in combination with the features of any claim to which it refers, meet the requirements of novelty and/or inventive step (see documents as cited in the search report).

Re Item VII

Certain defects in the application

4 **Minor objections**

4.1 Independent claims are not in the two-part form, which in the present case would be appropriate, with those features known in combination from the prior art being placed in the preamble and the remaining features being included in the characterising part.

Re Item VIII

Certain observations on the application

5 **Clarity**

Claims 1, 3, 6 and 8 are not clear.

5.1 The terminology and the signs shall be consistent throughout the application. This requirement is not met in view of the use of the expressions "substrate based microfluidics", "silicon based microfluidics" and "substrate (silicon)" for the same feature (see claim 1).

5.2 The term "silicon based" used in claim 1 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear. PDMS, for example, is silicon based, and therefore enters the scope of claim 1.

5.3 Claim 3 refers to a membrane which has not been previously mentioned in the claims on which claim 3 depends. Therefore, the subject-matter of claim 3 is unclear.

5.4 The term "further microfluidic elements" used in claim 6 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear.

5.5 The term "aligned with respect to the device" used in claim 8 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claim unclear. Such a wording does not allow the reader to understand to what part of the device, the micro-feature should be aligned.

5.6 The terminology and the signs shall be consistent throughout the application. This requirement is not met in view of the use of the expressions "first membrane polymer layer", "first membrane layer" and "polymer layer" for the same feature (see claim 11).

5.7 Claim 11 is not supported by the description. Method step "e2" of claim 11 is not disclosed in the description (see page 7).

5.8 Claim 22 comprises all the features of claim 11 and is therefore not appropriately formulated as a claim dependent on the latter.

5.9 Claim 22 is not supported by the description. According to the description on page 12, the substrate optionally comprises a sensor, but the presence of a microelectrode array, or microgrooves is not mentioned.

5.10 Moreover, the method step "a2" includes patterning the dielectric layer on a top AND on a bottom side. The option of patterning only on one of the two sides, is not disclosed in the description.