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(54) **Titre : MICRO-ENVIRONNEMENT EXTENSIBLE 3D POLYVALENT POUR DISPOSITIFS D'ORGANE-SUR-PUCE FABRIQUES A L'AIDE D'UNE TECHNOLOGIE DE SILICIUM STANDARD**

(54) **Title: VERSATILE 3D STRETCHABLE MICRO-ENVIRONMENT FOR ORGAN-ON-CHIP DEVICES FABRICATED WITH STANDARD SILICON TECHNOLOGY**

(57) **Abrégé/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 o the device as a microreactor.

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 o 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 micro-reactor.

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}-

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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, and WO2016/004394 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.

Recently one of the present inventors published an article (Gaio et al., “Cytostretch, an Organ-on-Chip Platform”, Micromachines, Vol. 7, July 14, 2016, 120 (p. 1-14). The Cytostretch device does not relate to a microfluidic channel. It is a foil that does not have any channel. For fabrication some steps overlap with the present method, but the above patent WO2015/138034 A1 and this paper do not relate to e.g. a channel in a thin layer.

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.

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SUMMARY OF THE INVENTION

The present invention relates in a first aspect to a device as disclosed herein, 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, also referred to as a foil, which is considered to relate to a selective barrier; in addition or in alternative, the present polymer layers may be considered to relate to a film, i.e. a thin continuous polymeric material, whereas a thicker plastic material would relate to a sheet; 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, micro-particles, 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; the top layer is optically transparent, or at least largely transparent, in order to use a microscope to view samples, such as biological cells, such as >90% transparent e.g. > 98%; 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

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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, such as 300-350 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 first micro-chamber(12) embedded in the substrate is accessible from the outside; in an example it may be regarded as a cavity, having a bottom and one or more side walls. The present polymer is independently selected from biocompatible polymers, such as polysiloxanes, such as polydimethylsiloxane (PDMS), polyimides, polyurethane, styrene-ethylene-butylene-styrene (SEBS), polypropyl-

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ene, 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 or foil, 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 macro-chamber can have dimensions in the order of 100*100 μm^2 to 10*10 mm^2. 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) provided 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

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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; Microgrooves 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 hotplate 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 stimulations and also stratified structure; simulation of a microenvironment 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, trans-endothelial 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 microenvironment 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

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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, micro-channels and micro-chambers of the present device may be used to develop microfluidics devices and/or micro-reactors 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, i.e. on one side only; then depositing a first foil 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 polymer membrane layer 61 is patterned using a lithography or electron beam machine; the first membrane polymer layer

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is etched with plasma etching/dry etching; 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 36 to stretch the membrane and an electrical output 18 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.

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DETAILED DESCRIPTION OF THE INVENTION

In an example the device of the present invention comprises at least one of a microchip, an integrated sensor, and an output 18, such as embedded in the polymer based microfluidics and/or integrated and embedded in the substrate based microfluidics. 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 polymer 20a-b (foil) 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 poly-siloxanes, such as polydimethylsiloxane (PDMS), polyimides, polyurethane, styrene-ethylene-butylene-styrene (SEBS), polypropylene, polycarbonate, polyester, polypropylene, butyl rubber, ostemer, 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 polymer top layer (or 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

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as m∈[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 of the present device the polymer top layer (or membrane) comprises a plurality of interconnected hollow structures, such as a scaffold-like structure.

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 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 um, such as 1-500 um, a width between 0.4 to 5000, such as 1-50 um, and a depth between 0.2 and 50 µm, such as 1 to 20 um, e.g. 2-5 um.

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

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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 an edge of the device or with respect to the first micro-chamber; the alignment may for instance be parallel to the edge or perpendicular thereto; the micro-features are typically aligned with respect to one and another as well, e.g. in a parallel fashion.

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 micro-chamber 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 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, 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.

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In an example of the present device the walls of the micro-chamber 21 (cavity-like structure) and/or 12 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 for making one or more devices, such as in a semiconductor process-like environment.

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 foil 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 foil 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 foil layers 61,62 are each independently made from a material selected from a biopolymer, preferably a biocompatible polymer, such as poly-siloxane, 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

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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 foil layers is spun.

In an example and/or partly in an alternative of the above method for making one or more devices, such as in a semiconductor process-like environment, the present method may comprise at least one step selected from:

providing a Si-substrate 10, optionally comprising at least one sensor 90, a microelectrode array 29, and at least two sets of micro-grooves 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 top and/or bottom side, i.e. on one of either sides or on both of the sides;

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

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set of micro-grooves;

f2) patterning the first sacrificial layer 71;

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

h) patterning the first foil 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

j1) depositing a second sacrificial layer 72 on the first foil layer, such as PR, and PR spinning and

j2) patterning the second sacrificial layer 72 using a lithography or electron beam machine;

k) depositing a second foil layer 62 on the sacrificial layer 72;

l) patterning the second foil layer 61;

m) 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;

n1) deposition of a conductive and/or insulating chamber coating 14, such as platinum parylene; and optionally a chemical surface treatment, such as a hydrophilic treatment,

n1) etching of the conductive or insulating chamber coating;

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

p) (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 lithog-

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raphy, 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 as discussed hereinafter.

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 micro-chamber 22. These cells may be cultured on the silicon micro-chamber 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 trans-endothelial 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 34 comprising an inlet 36, an electrical input/output 38 connected to a printed circuit board (PCB) 39 with at least one electrical output 18, e.g. via flip chip connections or wire-bonding 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

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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-p show details of an exemplary embodiment of the present method.

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

Figs. 13-16 show examples of the present device and features thereof.

DETAILED DESCRIPTION OF THE FIGURES

In the figures:

100 microfluidic device
10 substrate
11 first micro-channel
12 first micro-chamber (macro-chamber)
14 coating layer
16 input
18 output
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

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37 electrical connection
38 electrical input/output
39 printed circuit board
51a,51b 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-p 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)

Figure 1j shows sacrificial layer deposition and patterning (for channels); k) Second PDMS layer spinning; l) Second PDMS layer patterning; m) Silicon etching; n) Macro-chamber coating (platinum/parylene); o) Silicon oxide etching; and p) Micro-

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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 micro-chamber 12.

Figure 3a and 3b show respectively details of an exemplary embodiment of two devices equipped with a microchannel 21 and a micro-chamber 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.

Figures 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 micro-chamber 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

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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 21 (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 34 comprising an inlet 36 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 wire-bonding connections 37, in order to interface with the electrodes 29 and or the sensors 90 embedded in the device 100.

Figures 13a-c show an example of openings 28 etched in thin polymer top layer 20a. The holes have a circular shape and a width of about 5 µm (fig. 13a) and about 7 µm (figs. 13b-c), and a depth of about 5 µm. The holes are interconnected through the foil by passages of which a few are indicated with arrows. As such a very open scaffold type foil is formed, such as comprising a plurality of interconnected hollow structures.

Figure 14 shows an example of the present device held by a thumb and a finger. Therein substrate based microfluidics 10, first micro-chamber (macro-chamber) 12, which macro-chamber may be a cavity, and second micro-channel 21 can be seen.

Figures 15a-l show examples of size and pore-pore distance variations being possible with the present device. The top row has a pore size of 1 µm, the middle row of 2.5 µm and the bottom row of 5 µm. The left column has a pore-pore distance of 1 µm, the second row of 2 µm, the third row of 3 µm, and the right row of 4 µm.

Figure 16 shows two examples of the present device. In the top example in the substrate 10 microfluidics are provided. A

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first micro-channel 11, a first micro-chamber (macro-chamber) 12, which macro-chamber may be considered as a cavity (here and throughout the description as well) and an input 16 are provided. The height of the substrate is about 500 µm. In the polymer layer only one horizontal micro-channel 21 is shown. In the bottom example even less elements are provided. A width of the channel 21 is from 1-5 cm.

EXAMPLES/EXPERIMENTS

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

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CLAIMS

1. Micro-fluidic device (100) comprising

(a) polymer film (20a, 20b), the film comprising

a polymer top layer (20a) composed of optically transparent polymer and having a first thickness in a range of 0.05 µm to 30 µm, and

a polymer bottom layer (20b) composed of optically transparent polymer and in contact with the polymer top layer, the polymer bottom layer (20b) having a second thickness in a range of 50 µm to 2000 µm and comprising at least one of a second micro-channel (21) and a second micro-chamber (22) at least partly embedded in the polymer bottom layer,

(b) a rigid substrate (10) composed of silicon or dielectric silicon, the substrate being in contact with the polymer top layer (20a) and comprising

a first micro-chamber (12) forming a cavity that is embedded in the substrate, the cavity being bounded from below by the polymer top layer (20a) and being open and accessible from a top side of the micro-fluidic device via an access opening provided in an upper surface the substrate (10), and

a first micro-channel (11) embedded in the substrate, wherein the first micro-channel (11) is accessible via at least one input (16) provided in the upper surface of the substrate, the first micro-channel (11) forming a micro-fluidic interconnection between the input (16) and the at least one of the second micro-channel (21) and the second micro-chamber (22) embedded in the polymer bottom layer;

wherein the polymer top layer (20a) comprises a matrix of holes (28) thereby forming a selective barrier separating the first micro-chamber (12) from the at least one of the second micro-channel (21) and the second micro-chamber (22).

2. Device according to claim 1, wherein the device is formed as a microchip.

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3. Device according to claim 1 or 2, wherein the polymer is at least one of stretchable having a tensile strength of > 1 [MPa] (ISO 527), flexible with a Young's modulus of <3 [GPa] (ISO 527), and rigid having a Young's modulus of >10 [GPa] (ISO 527).

4. Device according to any one of claims 1-3, wherein the polymer is independently selected from biocompatible polymers, polyimides, polyurethane, butyl rubber, styrene-ethylene-butylene-styrene (SEBS), polypropylene, polycarbonate, polyester, and biodegradable polymers, and combinations thereof.

5. Device according to claim 4, wherein the polymer is a polysiloxane, Biorubber (PGS), poly(1,8-octanediol-co-citrate) (POC), polydimethylsiloxane (PDMS), off-stoichiometry thiol-ene polymer (ostemer), or a combination thereof.

6. Device according to any one of claims 1-5, wherein the matrix of holes (28) has a hole-to-surface density in a range of 0.001 to 250 per 100 μm^2, and wherein an average hole area for the matrix of holes is in a range of 0.05 μm^2 to 500 μm^2.

7. Device according to any one of claims 1-6, further comprising embedded in the device at least one of a sensor, a pump, a microelectrode, a valve, a strain gauge, 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 microelectrode array, a chemical stimulator, an optical stimulator, an ion sensor, and a pressure regulator.

8. Device according to any one of claims 1-7, comprising embedded in the polymer top layer (20a) at least one electrode (29), wherein the electrode has an accessible area (29a) of 0.1-5000 μm^2.

9. Device according to any one of claims 1-8, wherein the polymer top layer (20a) comprises at one side thereof an array of x*y microgrooves (27) that are aligned with respect to the first micro-chamber (12), the microgrooves having a surface

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density in a range of 1 to 25 per 100 μm^2 and an average groove area in a range of 0.1 μm^2 to 10^6 μm^2.

10. Device according to any one of claims 1-9, wherein the first micro-channel (11) has a height of 50-2000 µm, wherein the first micro-chamber (12) has horizontal dimensions in a range of 100*100 μm^2 to 10*10 mm^2, and the at least one of the second micro-channel (21) and the second micro-chamber(22) has a height of 1-1000 µm.

11. Device according to any one of claims 1-10, wherein the second micro-chamber (22) comprises at least one column (26) made of polymer, which connects the top and the bottom side of the second micro-chamber (22).

12. Device according to any one of claims 1-11, wherein walls of the first micro-chamber (12) and walls of the first micro-channel (11) are coated with an insulating layer or with a conductive layer (14).

13. Device according to any one of claims 1-12, wherein the polymer layers (20a, 20b) are provided with openings (18), the openings providing access to at least one of a metal pad, an IC, a sensor, and a heater.

14. Method for producing at least one device (100) according to any one of claims 1-13, the method comprising the steps of

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

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

B) patterning the dielectric layer on a bottom side;

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

D) patterning the first polymer layer (61) using a lithography or electron beam machine;

E) depositing a sacrificial layer (72) on the first polymer layer (61);

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

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

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

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

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

15. Method according to claim 14, wherein the first dielectric layer (51a, 51b) and a second dielectric layer (52) are made from a material independently selected from Si-dielectric materials.

16. Method according to claim 14 or 15, wherein a thickness of the first dielectric layer (51a, 51b), the second dielectric layer (52), and a third dielectric layer (53) are each independently from 10-50000 nm.

17. Method according to any one of claims 14-16, wherein a thickness of the first polymer layer (61) is from 50-30000 nm.

18. Method according to any one of claims 14-17, wherein a thickness of the second polymer layer (62) is from 50-2000 µm.

19. Method according to any one of claims 14-18, wherein the polymer layers (61, 62) are each independently made from a material selected from a biopolymer, polyimides, and parylene, and biodegradable polymers, and combinations thereof.

20. Method according to any one of claims 14-19, wherein dimensions of the at least one first micro-channel (11) and at least one first micro-chamber(12) embedded in the substrate, the at least one input (16), the at least one second micro-channel

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(21) and 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 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.

21. Device according to any one of claims 1-13, for use in 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 organ, as Lab-on-Chip, as a microfluidics device, and as a micro-reactor.

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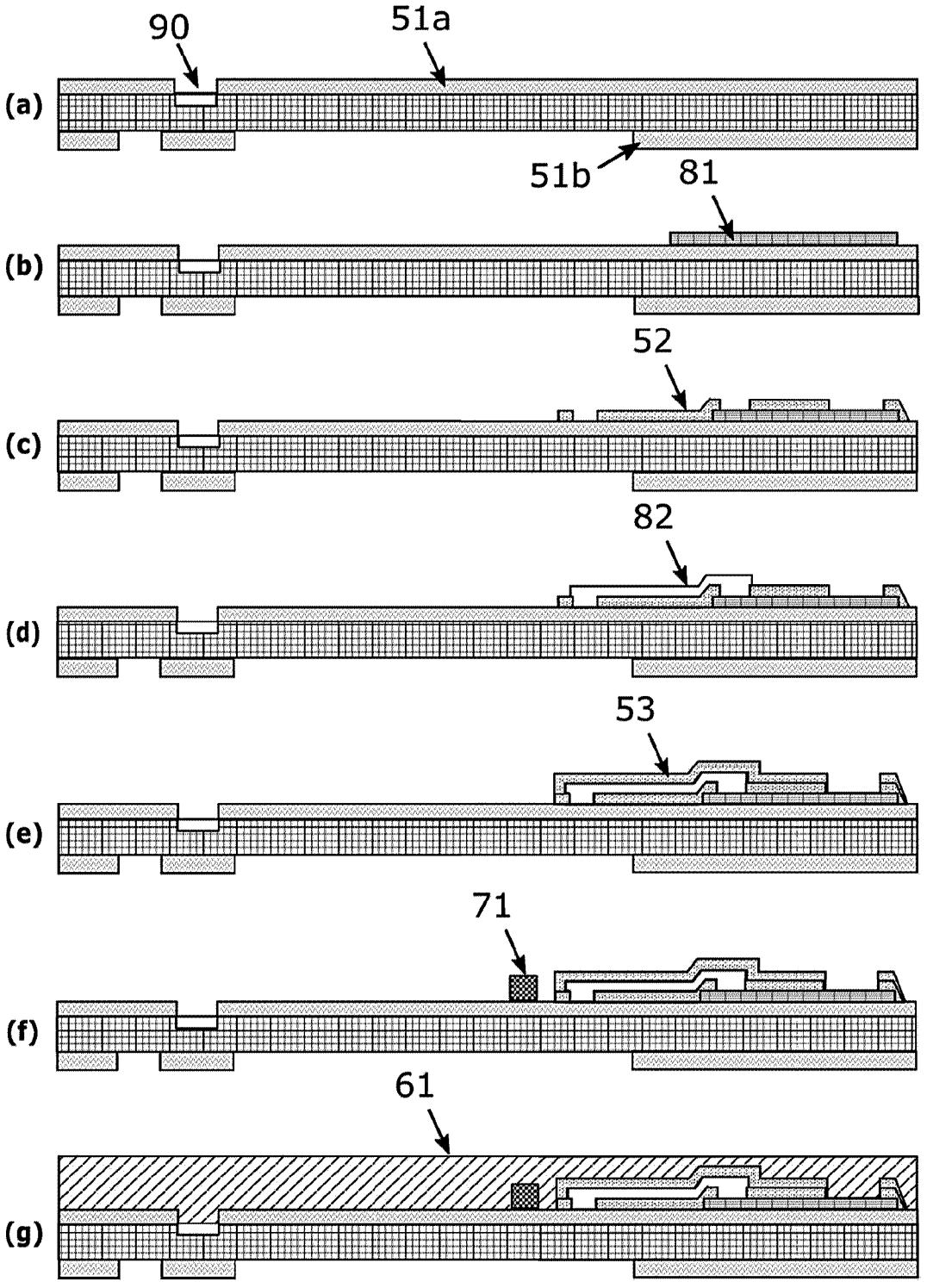

Fig. 1a-g

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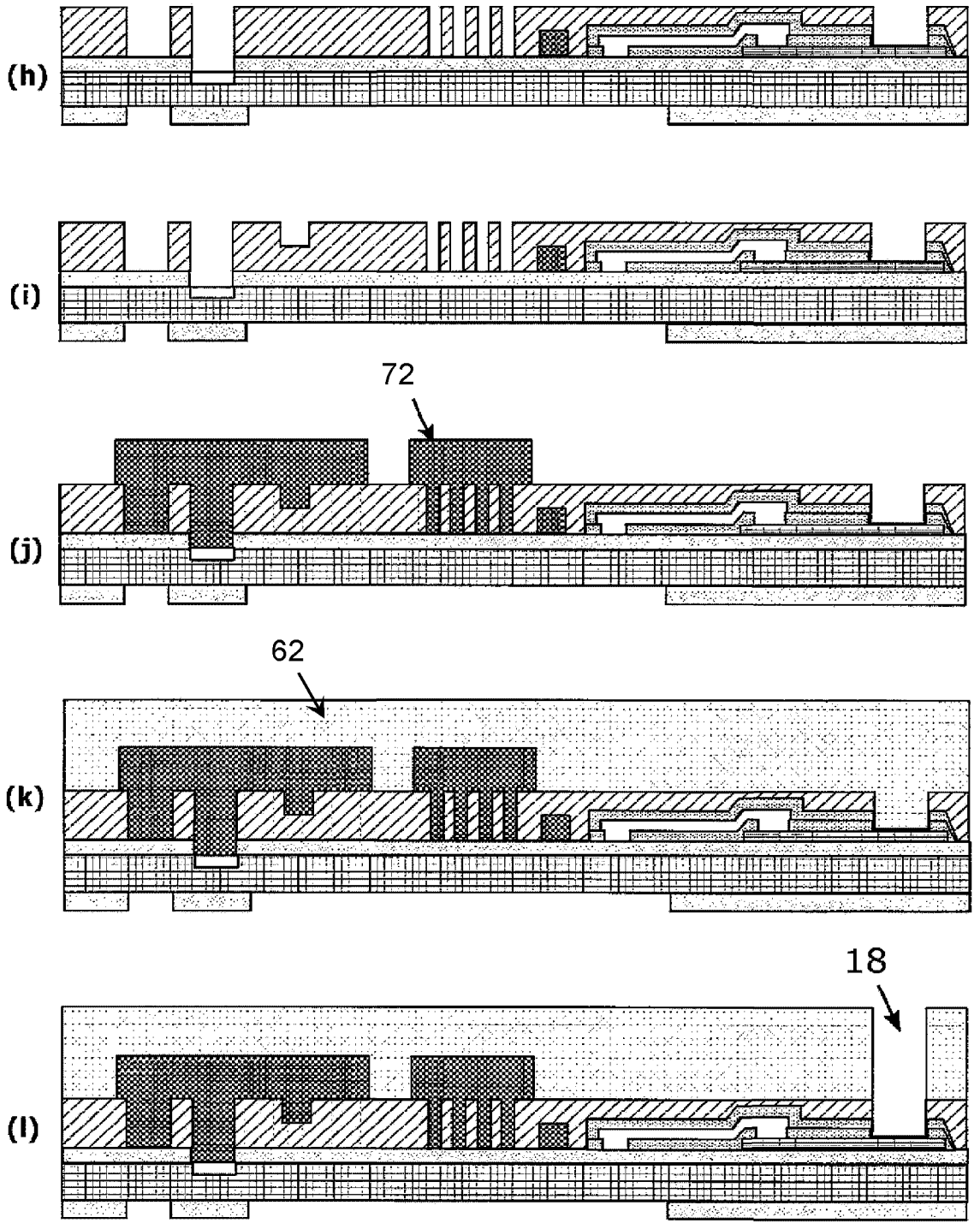

Fig. 1h-l

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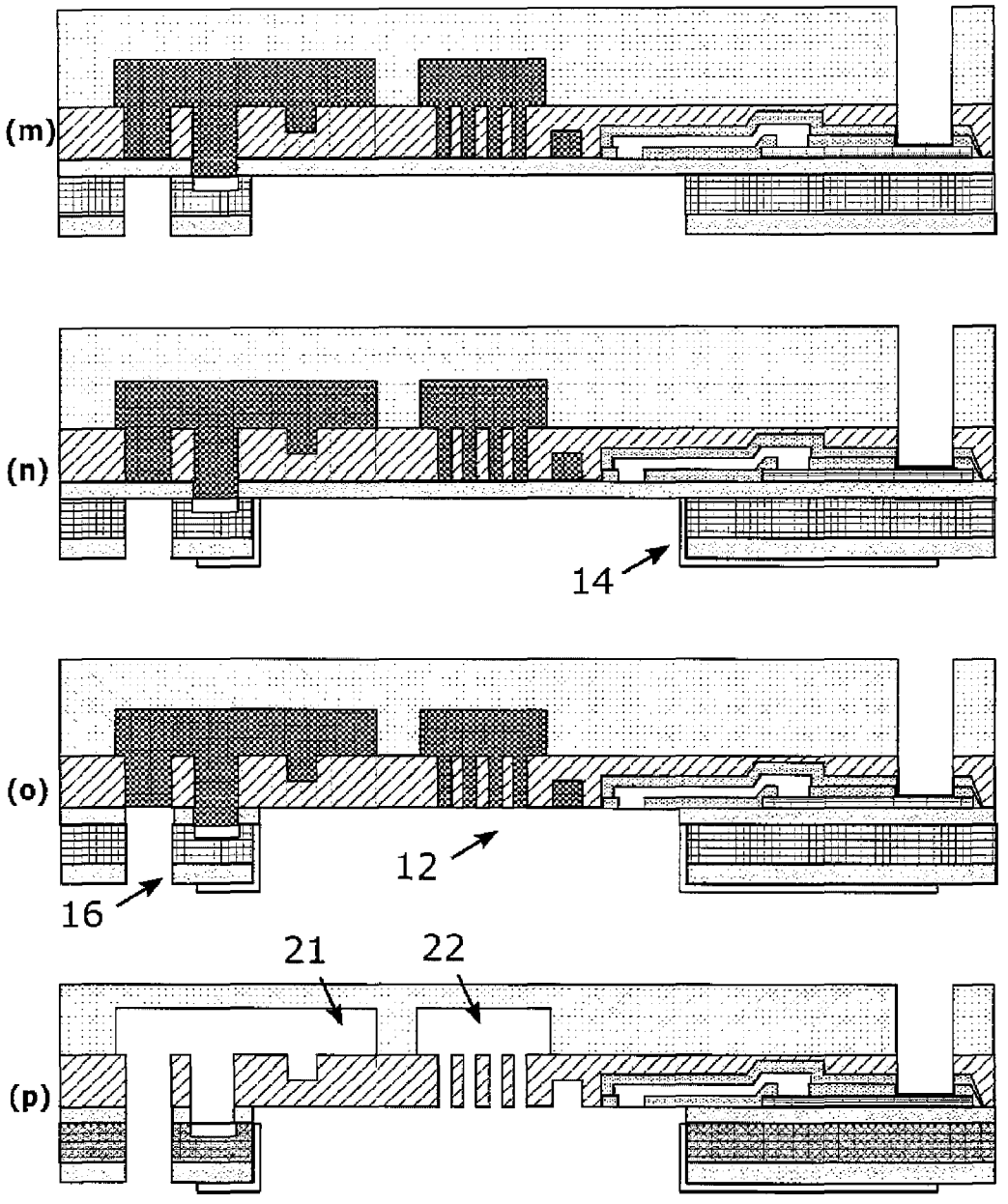

Fig. 1m-p

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Fig. 2a

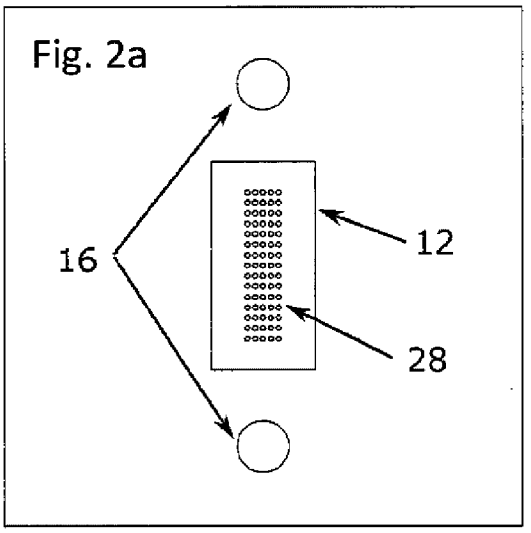

Fig. 2b

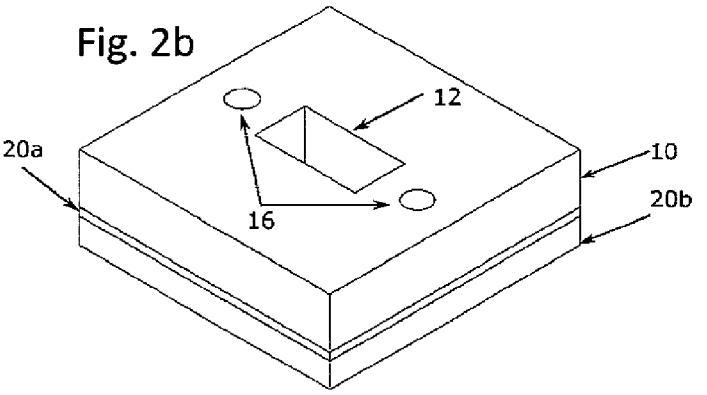

Fig. 2c

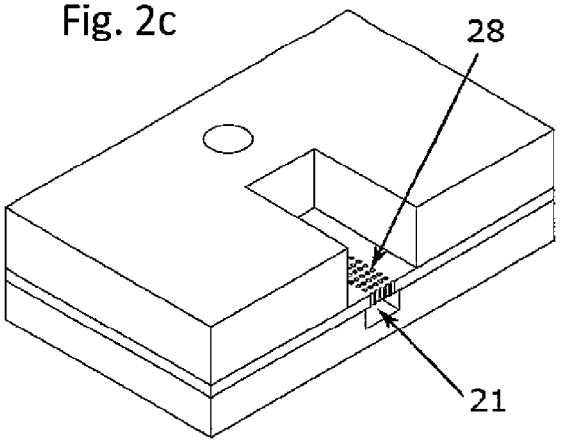

Fig. 2d

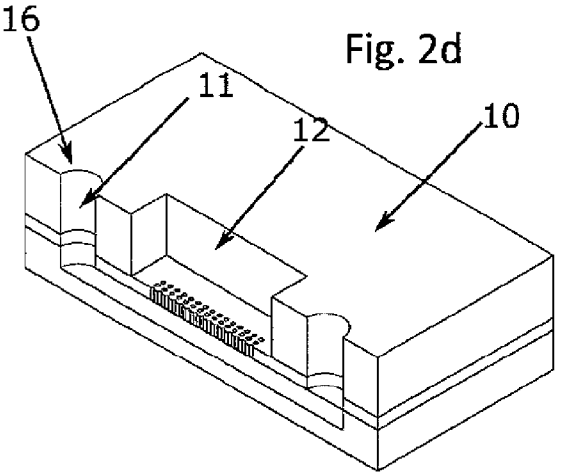

Fig. 3a

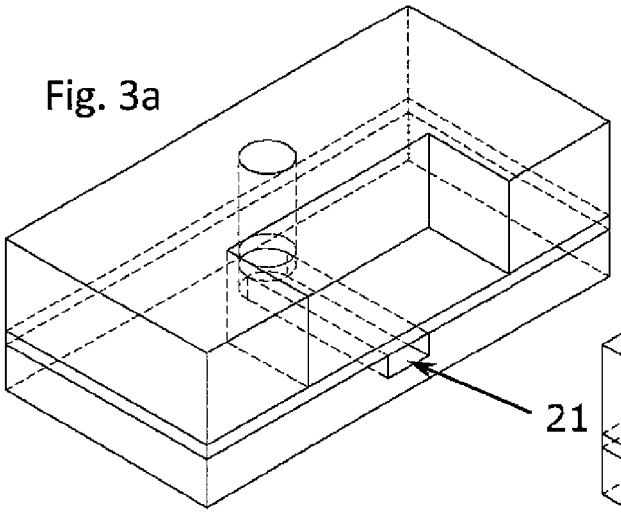

Fig. 3b

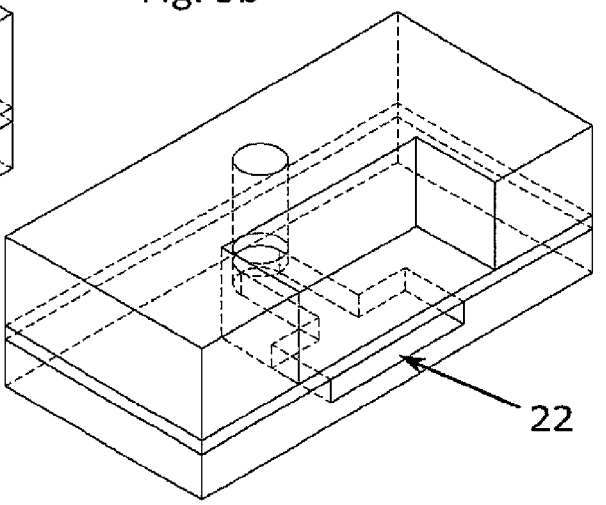

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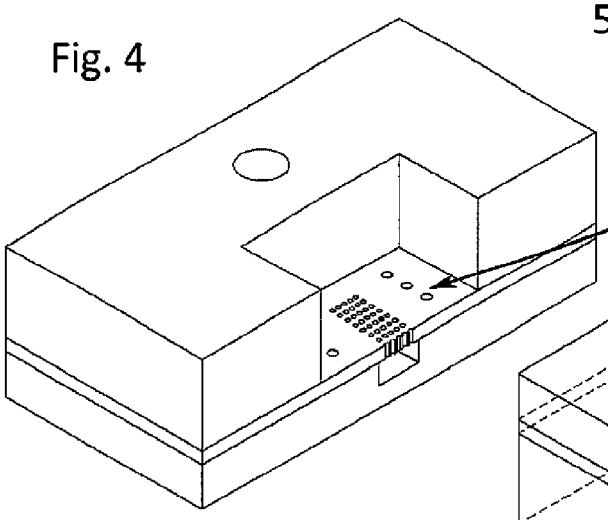
Fig. 4



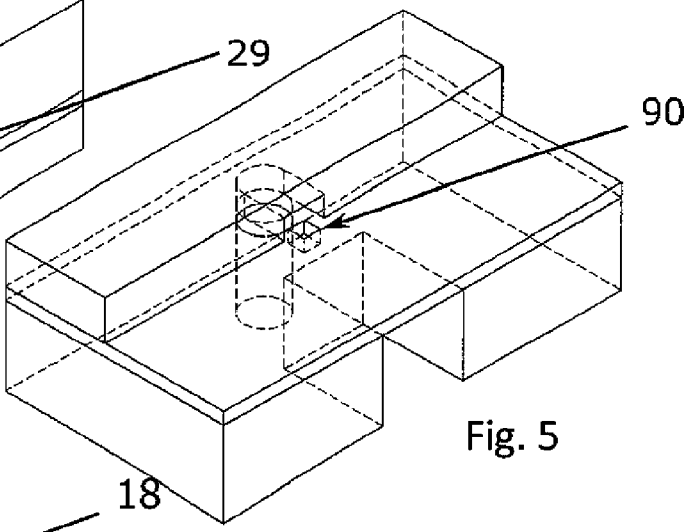


Fig. 5

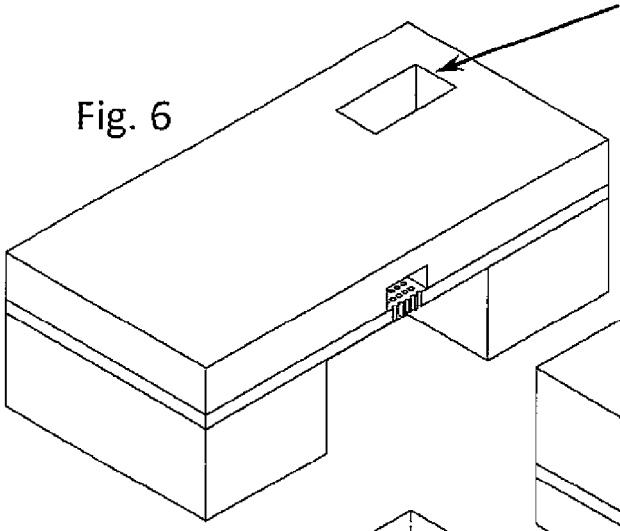


Fig. 6

Fig. 7

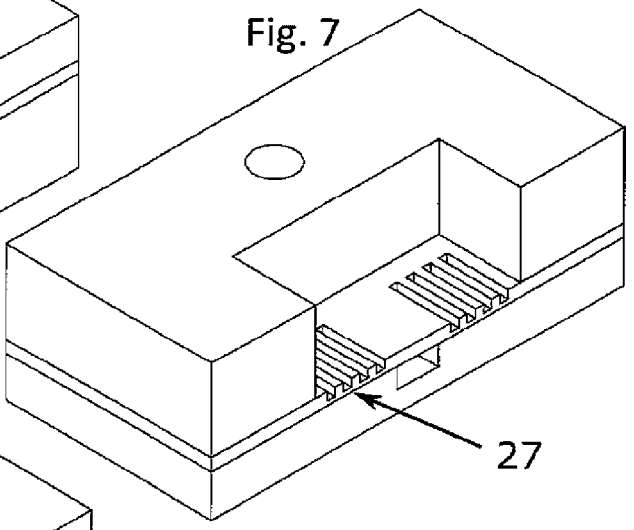


Fig. 8

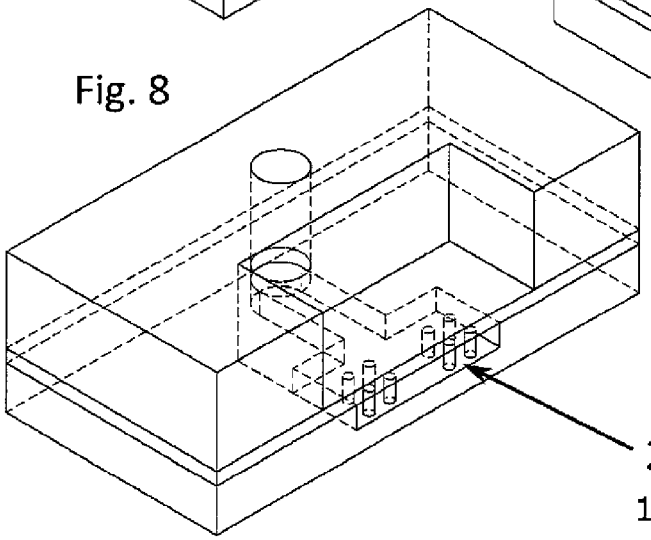
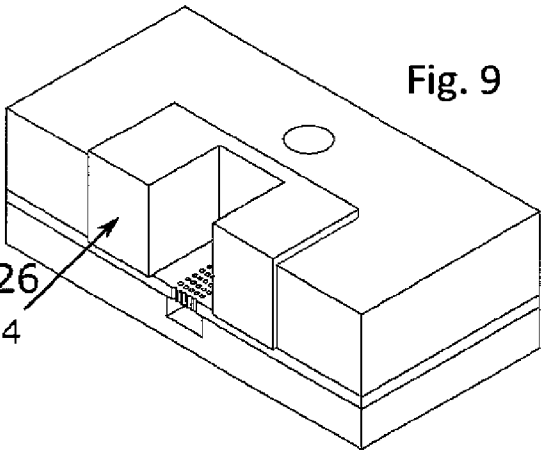


Fig. 9



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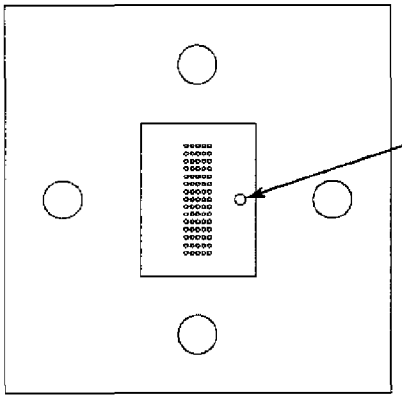

Fig. 10a

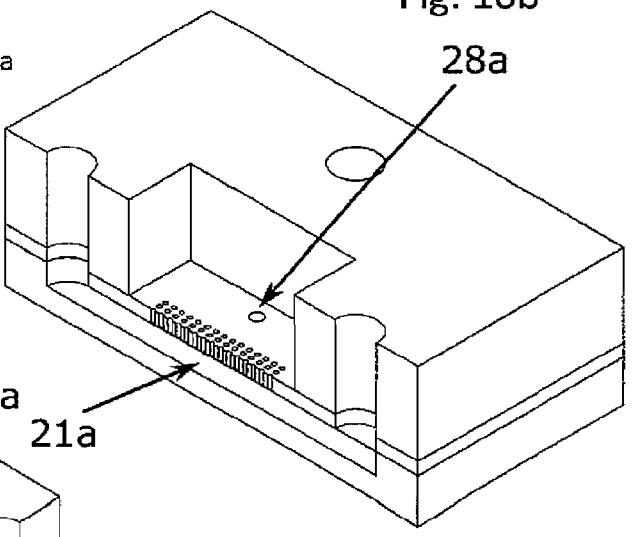

Fig. 10b

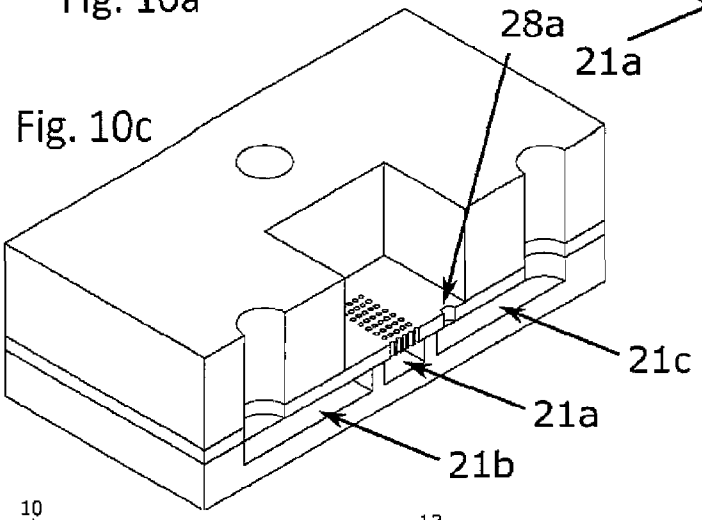

Fig. 10c

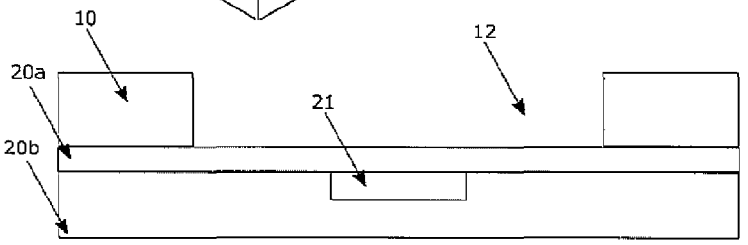

Fig. 11a

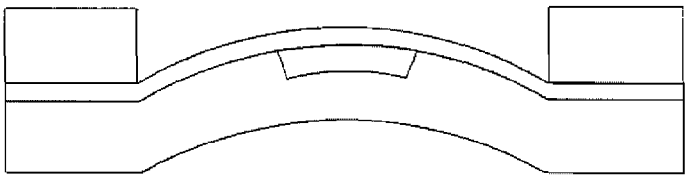

Fig. 11b

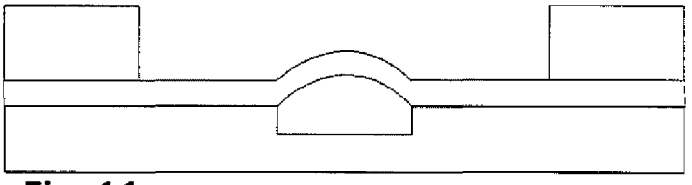

Fig. 11c

Fig. 11

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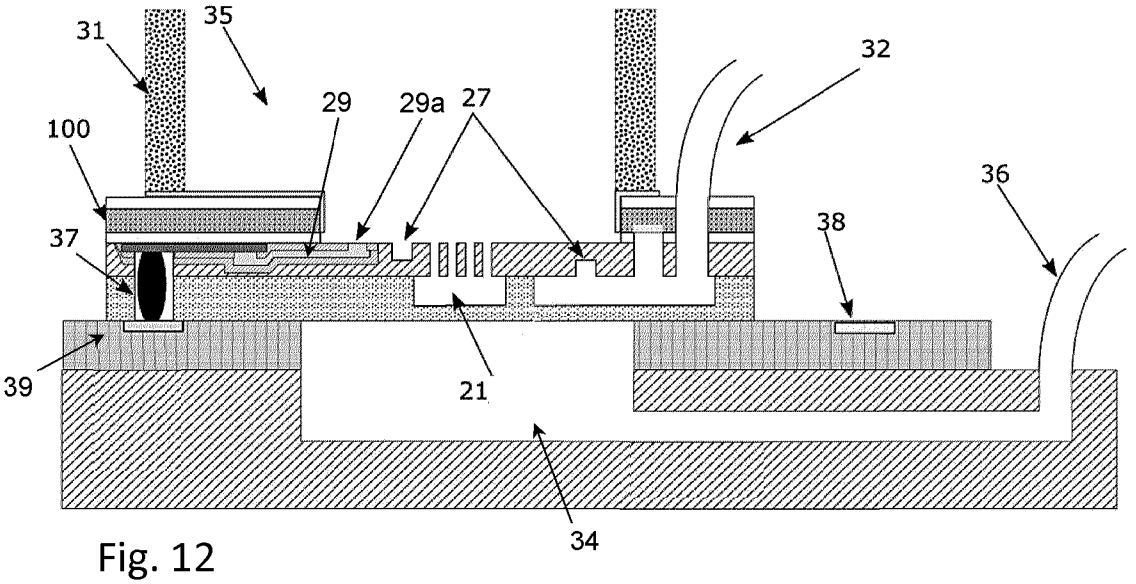

Fig. 12

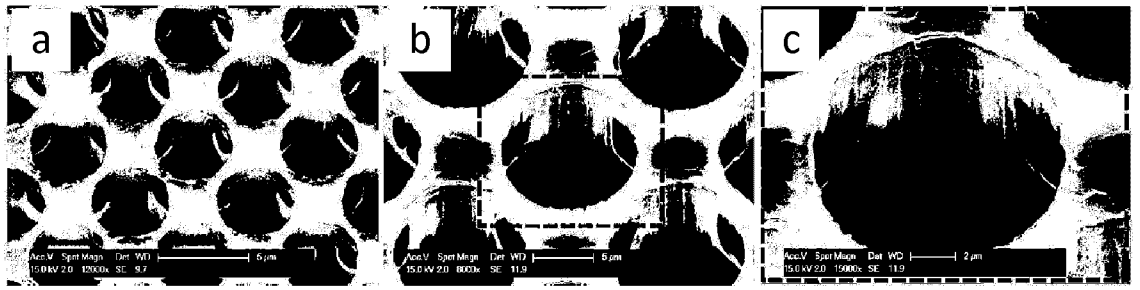

Fig. 13

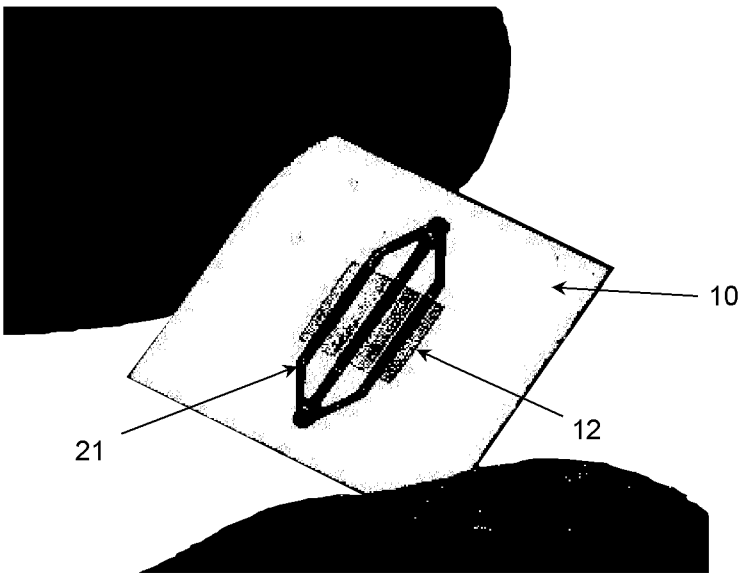

Fig. 14

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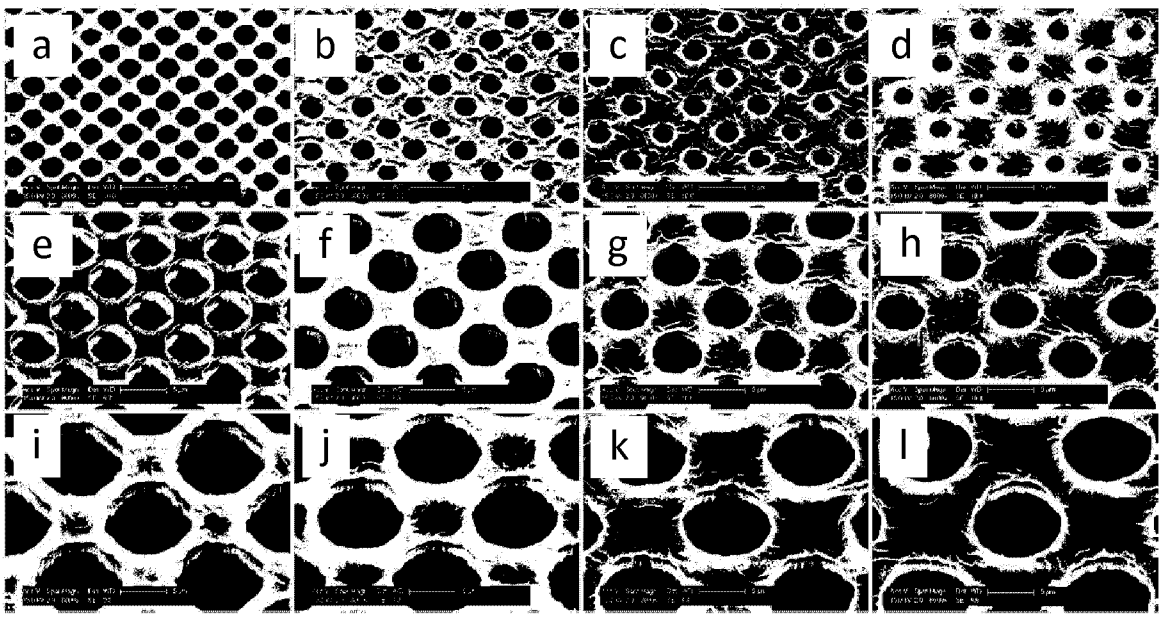

Fig. 15

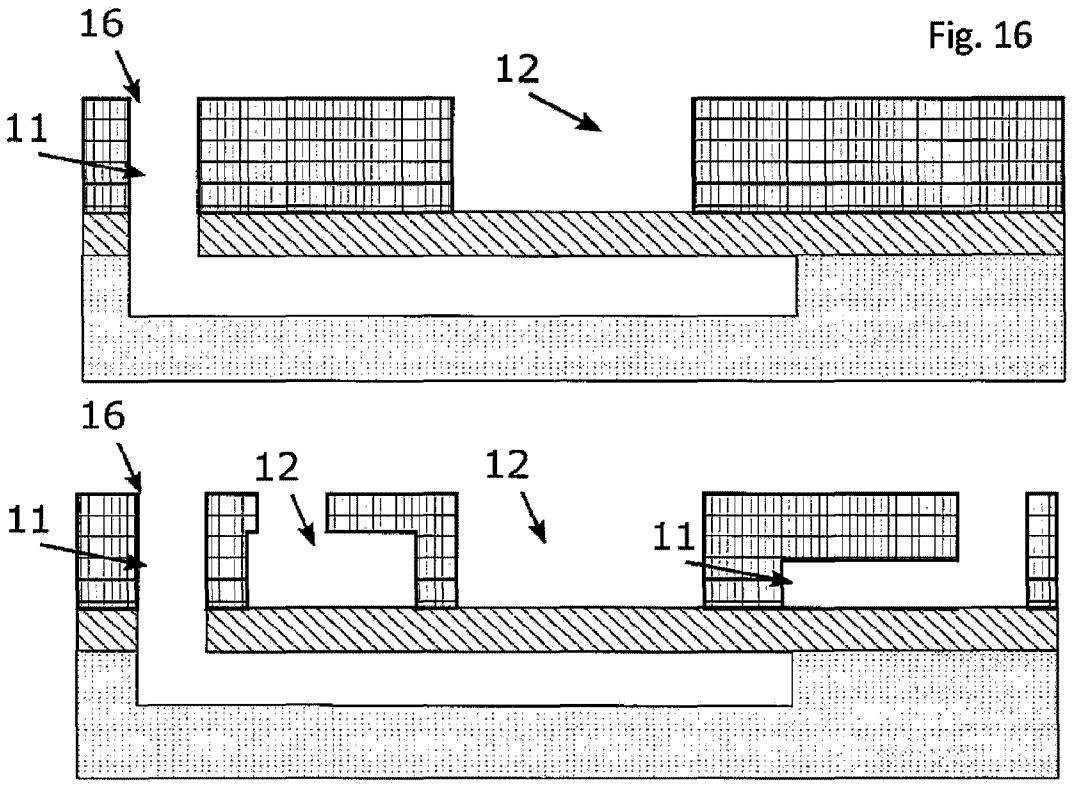

Fig. 16

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