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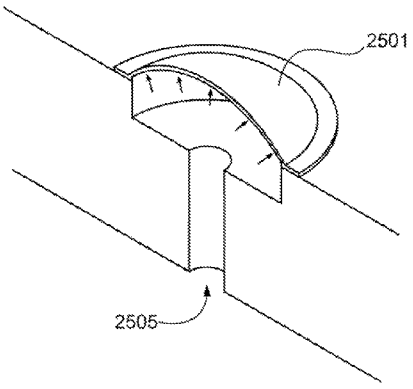
(54) Title: LOW ELASTICITY FILMS FOR MICROFLUIDIC USE

Fig. 1B



(57) Abstract: Microfluidic circuit elements, such as a microvalve, micropump or microvent, formed of a microcavity divided by a diaphragm web into a first subcavity bounded by a first internal wall and a second subcavity bounded by a second internal wall, where the diaphragm web is characterized as a thin film having a first state contacting the first internal wall and a second state contacting the second internal wall and exhibiting essentially no elasticity in moving between the first state and the second state, the thin film web having been stretched beyond its yield point before or during use are provided. The disclosed elements enable faster and more efficient cycling of the diaphragm in the microcavity and increases the diaphragm surface area. In a preferred embodiment, the microfluidic circuit element is pneumatically driven and controls the motion of fluids in a microassay device.

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LOW ELASTICITY FILMS FOR MICROFLUIDIC USE

BACKGROUND

Technical Field

This disclosure is generally directed to diaphragm technologies for microvalves, micropumps and other pneumatic fluidic elements for use in microassay devices, and to their methods of manufacture.

Description of the Related Art

Microassay cartridges have found increasing use as devices for diagnostic assays. The devices described by Wilding in US Patent No. 5,304,487 consisted of "mesoscale" channels and chambers formed on reusable silicon substrates that were infused with fluid reagents from off-cartridge syringe pumps. Little consideration was given to on-board fluid handling and control. However, practical commercial applications have led in the direction of "consumable" cartridges—disposable, single use "sample-to-answer" cartridges that are self-contained for all reagents needed for a particular assay or panel of assays.

Microscale means for handling fluids include mechanical hydraulic systems such as piston driven devices, electrical hydraulic systems such as electrokinetic pump and valve devices, and pneumohydraulic systems. Of these, those systems with pneumatic actuators and control surfaces have proven to be particularly practical in controlling microscale fluid flows.

One well known class of fluidic devices having a pneumatic interface is manufactured by the Assignee, Micronics, Inc. (Redmond, WA). Control of fluid flow in microfluidic channels is achieved with a MICROFLOW® system pneumatic controller that operates miniature valves in a plastic cartridge according to programmable valve logic. Diaphragms separate the pneumatic side and the hydraulic side of the cartridges; *i.e.*, the valve diaphragms are interface elements for converting pneumatic control pulses into starting and stopping fluid flow. Cartridges are formed by building up laminations, layer by layer, with channels and chambers sealed between capping overlayers. In this way, complex fluidic circuits are formed.

To form a fluidic circuit by conventional fabrication, a layer of an elastic material is sandwiched as a laminate between body layers, and pneumatic and hydraulic channels and chambers are formed in the apposing layers on either side of the elastic layer, such that the pneumatic workings and the hydraulic workings of the cartridge are

separated by a diaphragm layer. Diaphragms formed of polyurethane, polyimide, and PDMS have been favorites for this method.

Miniature pump elements, for example, are needed to achieve the fullest benefit of fluidic microcircuitry technologies, which find numerous applications such as in diagnostics and in life sciences more generally. Diaphragm-driven pumps are advantageous because of the absence of mechanical seals and lubricant, and their sanitary features.

Although miniature pumps were generically hinted at by Wilding (for example in US Pat. Nos. 5304487 and 5498392), the disclosures themselves were not sufficient to enable fluidic microcircuitry pumps and valves. Cited by Wilding was Van Lintel [1988, "A Piezoelectric Micropump Based on Micromachining of Silicon," Sensors and Actuators, 15:153-167], which relates to silicon-based microelectromechanical (MEMS) structures. However, silicon is known to have a very high Young's modulus (about 100GPa); therefore a silicon diaphragm pump will generally have a very low compression ratio ε defined by:

$$\varepsilon = (\Delta V + V_0)/V_0$$

where ΔV is the stroke volume and V_0 is the deadspace volume, *i.e.*, the volume of fluid that is not displaced from the pumping chamber during an ejection stroke. Thus disadvantageously, these devices may not be self-priming in operation when used with liquids.

Representative art related to siliceous diaphragm pumps includes U.S. Pat. Nos. 5759014, 6390791 and 7749444. Similar issues are seen with the rigid polymeric diaphragm members of US Pat. No. 7832429 and more generally where the diaphragm member resists deformation due to mechanical stiffness.

There has been greater interest in elastomeric diaphragm materials because of the higher compression ratio, which offers the advantage of self-priming in fluidic operations, and larger displacement volume. For example, polydimethylsiloxane (PDMS) and silicones may be used as diaphragm materials. Latex rubber and amorphous polyurethanes have also been used. Elastomeric materials that obey Hooke's law have the advantage that the diaphragm returns to its original shape in the relaxed state, but this is advantageous only for some applications, and can be associated with reduced chemical resistance and increased permeability.

Representative art related to microvalves includes U.S. Patent No. 4304257 (the '257 valve), in which a soft, resilient, polyurethane sheet is clamped over flow channels formed in a hard acrylic body. A fluid path between two discontinuous fluid channels is opened and closed by actuating pistons which mechanically flex a part

of the sheet. A tenting action on the sheet is associated with valve opening; valve closing is associated with spring return of the resilient sheet to a closed position. The sheet is flexed mechanically between the two positions by a solenoid-operated rod having an embedded attachment to the sheet over the valve seat, such that the sheet contacts the seat when closed and the sheet is pulled into an aperture overlying the valve seat to open the valve.

According to the teachings of US Pat. No. 4848722, the '257 valve has several disadvantages. In addition to delicacy of mechanical solenoid operation and need for fine adjustment, the membrane is subjected to great stresses with the risk of permanent stretch (*i.e.*, permanent deformation or pinching past its yield point). By virtue of the concave contact surface for the membrane, the sealing area is maximized, but disadvantageously, a non-zero and significant volume of the valve cavity must be filled before fluid begins to flow.

In expired U.S. Pat. No. 4848722 (the '722 valve), a pressure or vacuum source is used to urge a flexible sheet such as biaxially oriented polyethylene terephthalate (BoPET) into a stop-flow position in which apertures formed by the channels (3,4) in the valve seat are closed and an open position in which the apertures are fluidly confluent. The step land (Fig. 9: 62) of the valve seat is contacted by sheet (8) when the valve is closed. The sheet is glued to the pneumatic side of the valve.

U.S. Patent No. 4869282 describes a micromachined valve having a diaphragm layer sandwiched between two rigid layers forming the valve cavity. The diaphragm layer is formed of polyimide and is deflected by an applied pneumatic pressure in a control circuit to close the valve. Diaphragm motion is limited to avoid overstressing the polyimide layer.

Expired U.S. Pat. No. 5660370 (the '370 valve) describes a valve (FIG. 1: 1) having flexible diaphragm (2) and flat valve seat formed of a rigid layer in which two holes are formed, each hole defining an opening to a fluidic channel (3,4) in an underlying layer, where the holes are separated by a valve sill. The diaphragm is made of polyurethane or silicone. The valve (5) is opened by pneumatically exercising the diaphragm. To avoid the tendency of the sheet to become stressed beyond its yield point, a flat valve seat is used to minimize the required range of diaphragm motion. This also reduces the deadspace volume of the valve.

A similar structure is seen in U. S. Patent No. 5932799 to YSI Inc., which teaches a fluidic microcircuitry analyzer having a plurality of polyimide layers, preferably KAPTON® film, directly bonded together without adhesives and a flexible pneumatically actuated diaphragm member for controlling fluid flow.

WO Publ. No. 2002/081934 to Micronics, Inc., published Oct 17, 2002, describes a laminated valve having an elastomeric diaphragm. These valves, which were termed "peanut valves", admit fluid across the valve sill under negative pressure, and are closed when positively pressurized. Advantageously, the valve cavity is formed with a contoured waist to minimize deadspace volume.

US Pat. No. 7445926 to Mathies describes a laminate with a flexible diaphragm layer sandwiched between hard substrates. Pneumatic channels and fluid channels are formed on opposite sides of the diaphragm layer (*cf.*, fig. 1 of the reference), so that the diaphragm is the active valve member. The diaphragm material disclosed is a 254 micrometer PDMS membrane. The valve body is typically a solid such as glass.

US Pat. Appl. Nos. 2006/0275852 and 2011/0207621 to Montagu describe a fluidic cartridge for biological assays. The cartridge includes a molded body defining flow passages. A latex diaphragm and a canned diaphragm pump are shown (cf., fig 5 of the reference). The "rolling elastic diaphragm pump" member (3) is inserted into the cartridge as a pre-formed subassembly and is commercially available (Thomas Pumps, Model 1101 miniature compressor, Sheboygan, Wis. 53081). Valves are mechanically actuated using a stepper motor. Thus the valves have the disadvantage of requiring sensitive and meticulous adjustment for proper operation.

Other elastomeric valve and pump constructs are known. Examples of silicone valve construction include U.S. Pat. Nos. 5443890, 6793753, 6951632 and 8104514, all of which illustrate soft lithographic processes (*cf.*, U.S. Pat. Nos. 7695683 and 8104497) for forming valves and pumps. PDMS may be used to form diaphragms and pump bodies. Latex rubber and amorphous polyurethanes have also been used as diaphragm materials, but chemical resistance may not be sufficient for some applications.

Diaphragm members having toughness, solvent resistance and capable of being shaped into yield-in-place diaphragms have not previously been demonstrated. Advantageously, a solvent-resistant diaphragm that yields to form a pre-shaped diaphragm member has application in pumps and valves used for pumping suspensions of particulates, and for replacing elastomeric diaphragms such as polyurethane which may leak when exposed to caustics, chaeotropes, or solvents, thus permitting use of solvents such as ethanol, formamide and dimethylsulfoxide, e.g., for lowering the operating temperature requirements during PCR. Yield-in-place diaphragms have increased pump stroke ejection volumes, leading to faster circuit response, and improved flow of particulate solutions, such as bead slurries, for example. Although

progress has been made, there is a need for improved diaphragm construction of microassay cartridges, and in particular for a process applicable to miniaturized circuit elements. The present invention provides these and related advantages.

BRIEF SUMMARY

To improve the efficiency and speed of operation of a microfluidic circuit element such as a valve or a pump, it is desirable that the work required to change from a first state to a second state is minimal. A preferred class of circuit elements is diaphragm operated. The pneumatically controlled diaphragm separates a pneumatic subcavity from a hydraulic subcavity and operates on a fluid contained in the hydraulic subcavity. The diaphragm "web" is a thin film that serves as a barrier between the two subcavities, dynamically translating pneumatic pressure into fluid motion, or stasis. In a first state, the diaphragm web is in a first position between the subcavities, in a second state the diaphragm web is displaced from the first position and occupies a second position. Generally the first position conforms proximately to an interior surface of the hydraulic subcavity and the second position conforms proximately to a second interior surface of the pneumatic subcavity, and by exerting a force, the diaphragm may be reversibly transitioned between the two positions or states.

Unfortunately, currently existing microfluidic diaphragms are generally elastomeric in nature and require overcoming the significant elastomeric resistance to change from a first state to a second state. Therefore, it is desirable if the work required to change from one state to the other was reduced. We have invented a novel barrier that has a substantially zero work function to change from the first state to the second state. This is accomplished by the use of a web having near zero elastomeric properties and a surface area significantly larger than the microcavity in to which the web is sealed. Most preferably, the surface area of the diaphragm web closely approximates the interior surface area of a subcavity, or if the subcavities are not symmetrical, then the surface area of the web closely approximates the interior surface area of the larger of the subcavities. The diaphragm web is thus a movable film having low elasticity for separating a hydraulic and a pneumatic subcavity of a microfluidic circuit element, where the area of the film is larger than the greatest cross sectional area of the microcavity.

Film Properties

It is desirable for the web for use in these microfluidic cartridges be a film that is not significantly elastomeric and generally matches the interior surface area

of one subcavity the target microcavity, preferably the interior surface area of the larger subcavity of the microcavity. Most preferably, the film requires little or no work to transition from one state to another. The film is desirably in a flaccid state until the applied control pressure drives the film to one side or the other of a microfluidic cavity by inverting the film position. Preferably, the film can also be described as having near zero or zero restorative force toward a reduced area state. Most preferably, the film is a low or non-elastomeric film that does not significantly self-restore to a form with a surface area approximating the cross-sectional area of the cavity, and substantially matches the interior surface area of the subcavities without significant over or under pressure. Using a non-elastomeric film with a surface area matching the interior surface enables several advantageous and novel properties.

The use of low or non-elastomeric films as microfluidic components enables the production of valves, pumps and microfluidic features that have advantageous features. Notably through the use of low or non-elastomeric films, the restoring force of an elastomeric film does not need to be overcome as the film is moved from one side of the microcavity to the other. This reduction in force arises because the pneumatic control needs only overcome the inertia of the membrane and fluid, not the elastic spring force of the film plus the inertia of the membrane and fluid. This enables a faster cycling and/or cycling with reduced pressure, or both, of the membrane between one side and the other, e.g. from the open state to the closed state for a valve.

Manufacture

The yielded film in a microfluidic assembly can be formed by a number of methods. The films can be formed after assembly of the microfluidic cartridge by applying sufficient pressure to stretch the film over its yield point, or with a mechanical press for stretching the film into a cavity in the microfluidic assembly, or they can be stretched by the use of a punch and die prior to assembly, such as by a process of pre-stretching diaphragm webs in bulk. Depending upon the film and the manufacturing processes it may be desirable to form the films with one or more of these processes. After typical manufacture, some films are flexible but have substantially no elasticity in their range of motion. By the use of suitable dies and forming processes these films can be formed into shapes complimentary to the target microfluidic cavities. For example it is known in the art that a heated vacuum die can be used stretch films into macroscopic bubbles having a generally cylindrical shape. By the use of a suitable die, the films may be stretched on a microfluidic die before the film is aligned with the first

microfluidic assembly and then continuously bonded to said first microfluidic assembly. Through the use of continuous roll to roll processes, significant time and cost savings in manufacture may be realized.

To generate a film by a yield in place process, the surface area of the assembled film and the area of at least one portion of the microcavity be of a ratio that when a pressure difference is applied to the chip, the film is forced to match the interior surface area of the cavity and stretch the film beyond its yield point. This process may be accomplished during the cartridge assembly, or after assembly, when it may be yielded as part of the manufacture process, or by the initial operation of the microfluidic cartridge. Some films such as SARANEX® can be yielded in place with a relatively low pressure difference. To yield other films, it may be necessary to provide additional external pressures to ensure that the desired yield pressure does not cause a mechanical failure of the microfluidic assembly.

The films can also be yielded in place during manufacture. This may be accomplished through the pressure differential method described above. Alternatively, the yielding can arise through mechanical means. For example, a punch and a partially assembled microfluidic cartridge can be used. The film can be bonded to one side of the microfluidic assembly. After bonding the film, a die can be mechanically pressed into the film, driving the film into the void below. Alternatively, the film may be expanded into a die, and the expanded film is then transferred into microcavity void. Through the choice of a suitable die and pressure, the film can be stretched into a non-elastomeric state. For some films it may be desirable to perform the mechanical stretching at elevated temperatures. For some choices of film and manufacturing speeds, it may be desirable to form the yielded films prior to the assembly step. This can be accomplished through the use of a suitable molds or dies to form the complimentary pattern of stretched films in the larger carrier film. It may be advantageous to cut the resultant stretched film portions from the carrier film upon transfer to the microfluidic subassembly. This can be done either by kiss cutting with a die, or by a selective cutting with a laser film cutter.

The film can also be yielded prior to assembly. In this case, the assembly process needs to gather sufficient non-elastomeric film to line the surface area of the desired target cavity. This can be accomplished through the use of a punch and die combination, or by vacuum or pressure forming the film into a die, wherein the die has dimensions similar to the target microcavity. The film can then be positioned on the microfluidic subassembly and the non-elastomeric film transferred by a suitable change in pressure. Those skilled in the art can recognize that it may be advantageous

to insert manufacturing steps in the process, such as transferring the film and a perimeter to an adhesive layer, bonding the ungathered film with heat, pressure, or solvent, applying adhesive to the ungathered material, cutting a perimeter to create a bondable area for the yielded film, or combinations thereof.

When the film is yielded prior to cartridge assembly, manufacturing conditions that might be harmful to the microfluidic chip may be used. Specifically, it may be desirable for manufacturing reasons to use either pressure or thermal processing steps that may be incompatible with the microfluidic assembly, or reagents therein. By performing the yield process off of the microfluidic assembly, it becomes possible to use combinations of pressure and temperature that are relatively inaccessible to an assembled chip. This permits the use of polymers such as polyimide that have optimal process conditions that exceed the strength, and or desired temperatures desirable for plastic microfluidic cartridges. By utilizing these manufacturing techniques, those skilled in the art will appreciate greater flexibility microfluidic cartridge design and manufacture.

In all cases, it is desirable that the film be stretched sufficiently to irreversibly yield the material. Specifically, the stretch applied should exceed the yield point of the film to create non-elastomeric or very low elasticity film such that the yielded film has a surface area and shape complimentary to the inner surface of the microfluidic cavity into which it is assembled. It is known in the art that not all microfluidic cavities are symmetric with respect to the film layer, and in these cases, it may desirable that the surface area of the film match the larger of the two microcavities for most uses. For some uses, it may be sufficient to have the film match the surface area of the hydraulic or pneumatic side only. For example, it may be desirable with several microcavities in sequence to have the operating volume change be different amongst the microcavities. This can be readily accomplished by having an asymmetric division of the microcavity by the film.

Diaphragms of the invention form components of micropumps, microvalves, and microvents. A micropump is one such inventive combination, the micropump comprising a cavity having a first subcavity configured to receive a fluid; a second subcavity configured to be reversibly pressurized; a diaphragm interposed between and separating the first subcavity from the second subcavity; and, wherein the diaphragm is a polymeric thin film web having a yield point and is characterized by a permanently overstretched deformation of the web. The thin film web inelastically conforms in a first state to a first internal surface of the micropump cavity when pressurized and in a second state to a second internal surface of the cavity when

depressurized. The micropump is configured to pump a liquid according to a pump stroke defined by the reversible motion of the permanently overstretched deformation of the web between the first state and the second state as driven by pressurization and depressurization of the second subcavity.

A second combination is a microvalve, the microvalve comprising a cavity having a first subcavity configured with a valve inlet, a valve outlet, and a valve seat interposed between the valve inlet and the valve outlet, wherein the first subcavity is configured to receive a fluid; a second subcavity configured to be reversibly pressurized; a diaphragm interposed between and separating the first subcavity from the second subcavity, wherein the diaphragm is enabled to be reversibly deflected against the valve seat according to whether the second subcavity is pressurized or depressurized, thereby defining an "ON" position and an "OFF" position of the microvalve; and, further characterized in that the diaphragm is a polymeric thin film web having a yield point and is characterized by a permanently overstretched deformation of the web. The thin film web inelastically conforms in a first state to a first internal surface of the cavity when pressurized and in a second state to a second internal surface of the cavity when depressurized. The microvalve is configured to open and close by the reversible motion of the permanently overstretched deformation of the web between the first state and the second state as driven by pressurization and depressurization of the second subcavity. The invention also comprises combinations of the diaphragm elements as components of microfluidic circuits and devices.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIGS. 1A and 1B are renderings of a diaphragm member being stretched by a pressure applied within a sealed chamber. Whereas elastically stretchable materials return to a relaxed state, inelastically stretched materials undergo permanent deformation.

FIGS. 2A and 2B illustrate schematically two states of an inelastically stretched material that undergoes collapse when depressurized.

FIG. 3 depicts a stretched diaphragm film conforming to the interior wall of a concave chamber.

FIGS. 4A and 4B depict a chamber in cutaway view, the chamber having generally vertical walls and layered construction. Illustrated is the geometry of a diaphragm web in process of being stretched to conform to the chamber.

FIGS. 5A and 5B are perspective views of a cutaway section through a generally rectilinear chamber, showing progressive stretching of the diaphragm web to conform to the interior surface area of the upper chamber subcavity.

FIGS. 6A, 6B, and 6C are plan and elevation views of a stretched diaphragm member. FIGS. 6B and 6C depict a first state and a second state, where the first state is distended and the second state is contracted.

FIG. 7 is an exploded view of a diaphragm assembly in a chamber constructed by lamination. A diaphragm element having an apron is shown within the stack of layers.

FIG. 8 is an exploded view of a layered chamber where the diaphragm element is formed from a layer of the device.

FIGS. 9A and 9B illustrate the operation of a diaphragm film.

FIGS. 10A and 10B are plots of stroke volume before and after a cycle of stretching of a film past its elastic limit.

FIG. 11 is a stress-strain analysis for a co-laminate film of low density polyethylene / ethylene vinyl acetate/ polyvinylidene chloride, / ethylene vinyl acetate, and low density polyethylene.

FIG. 12 is a stress-strain analysis for a film of an elastomeric polyurethane.

FIG. 13 is a stress-strain analysis for a film of a high modulus material: polyethylene terephthalate.

FIG. 14 is a stress-strain analysis showing hysteresis in three serial stretch and relax cycles of a co-laminate film of low density polyethylene / ethylene vinyl acetate/ polyvinylidene chloride, / ethylene vinyl acetate, and low density polyethylene

FIG. 15 is a stress-strain analysis showing hysteresis in three serial stretch and relax cycles for polyethylene terephthalate.

FIGS. 16A and 16B are cross-sectional views of a microvalve structure, showing an “ON” and an “OFF” configuration of the valve diaphragm. The valve diaphragm member is formed by a process of inelastic deformation.

FIG. 17 is a cutaway view of a valve with inelastically deformed diaphragm.

FIGS. 18A and 18B are plan and elevation views of an overstretched diaphragm member for a fluidic microvalve.

FIG. 19 is an exploded view of a valve structure having an overstretched diaphragm.

FIGS. 20A and 20B are cross-sectional views of a microvalve structure, showing an "OPEN" and an "OFF" configuration of the valve diaphragm.

FIG. 21 is a perspective view of a diaphragm web of FIG. 20.

FIG. 22A illustrates the concept of valve latency. Data for a pre-stretched yield-in-place valve is compared to a conventional elastomeric valve in FIG. 22B. The latency time is reported in milliseconds.

FIG. 23 is a theoretical analysis of the relationship between a critical web dimension L_c and a process pressure required to achieve a defined overstretch.

FIG. 24 is an experimental study of overstretch behavior in a diaphragm valve.

FIGS. 25A through 25F are sequential views of steps in a first process of mechanically stretching a diaphragm film in place.

FIGS. 26A through 26F are sequential views of steps in a second process of mechanically stretching a diaphragm film in place.

FIGS. 27A and 27B illustrate a close-ended channel with breathable diaphragm for fluid loading and priming of a pump having a breathable diaphragm.

FIG. 28 is an exploded view of a close-ended channel with breathable diaphragm having a body constructed of layers.

FIGS. 29A and 29B are schematic views of a flow-through fluidic element with microporous film for gassing or degassing a liquid.

FIG. 30A, B and C are electron micrographs of the fine structure of a breathable microporous polyurethane film.

FIG. 31 illustrates a representative fluidic circuit having a combination of diaphragm-operated circuit elements of the invention.

FIG. 32 illustrates a microfluidic cartridge formed of pneumatic and hydraulic circuits containing microvalves and micropumps of the invention.

FIG. 33 tabulates parameters such as yield stress of high and low modulus films and is useful in guiding selection of diaphragm materials for yield-in-place valve applications.

DETAILED DESCRIPTION

Although the following detailed description contains specific details for the purposes of illustration, one of skill in the art will appreciate that many variations and alterations to the following details are within the scope of the claimed invention. The following definitions are set forth as an aid in explaining the invention as claimed.

Definitions

A "cartridge" is an analytical device designed for operation by insertion into a host instrument. The host instrument supplies the pneumatic pressure, pulses, and detection means for performance of the assay. The cartridge contains hydraulic works and pneumatic works, including microscale channels, cavities and chambers. Sample and reagent liquids are conveyed in a hydraulic network of the cartridge or card; fluid flow is controlled and driven by a pneumatic network that interfaces with the hydraulics at diaphragms spanning selected junctions, channels and chambers. Typically, the body of the cartridge or card is made of a flexible plastic and may be formed by lamination, molding or a combination thereof. Body plastics may include, but are not limited to, polycarbonate, polyethylene terephthalate, cyclic polyolefins, acrylates, methacrylates, polystyrene, polyimide, polysilicone, polypropylene, high density polyethylene, low density polyethylene, graft and block copolymers, and composites thereof. A preferred cartridge is made from rollstock and includes dry reagents printed thereon. Other such cartridges may include molded body elements.

"Hydraulic works" of a device: includes the network or networks of intercommunicating channels and chambers that are intended to be wetted by sample or liquid reagents in the course of an assay. The hydraulic networks are configured with fluidic subcircuits for performing the steps of an assay.

"Pneumatic works" of a device: includes the network or networks of pneumatically actuated valves, pumps and diaphragms and interconnecting circuitry and manifolds that are useful for powering and controlling the hydraulics of the device. The pneumatic works of the cartridge device interface with positive and negative pressure sources on the host instrument and with valves, diaphragms, pumps and other pneumatically actuated elements that control and drive liquids in the hydraulic network.

While it may be said that the pneumatic works of the device are preferably operated with a gas such as air or nitrogen, it is also conceived that equivalent "pneumatic" circuits may be operated with a fluid more generally, where fluid is selected from a gas or a liquid, including liquids such as silicone oils, vegetable oils, fluorocarbon liquids, and the like. Thus in one variant of the invention, the pneumatic works are operated with a "fluid" having the characteristics of a liquid and the operation of the device is otherwise equivalent, as would readily be understood by one skilled in the art.

"Fluidic works" of a device: include the hydraulic works formed of a network or networks of internal channels and chambers wetted in the course of the assay and the pneumatic works formed of control and pump driving circuits powered by

positive and negative pressure sources derived from a host instrument via a pneumatic interface.

The fluidic works may be divided into fluidic subcircuits, where each subcircuit comprises channels and chambers for performing a particular function on a liquid sample or reagent. The fluidic subcircuits may be organized into serial subcircuits (such as for extraction, amplification and detection of a nucleic acid target or targets) and parallel subcircuits and networks such as for simultaneous assay for multiple targets on a single sample by splitting the sample. "Microscale" and "fluidic" refer to devices having submillimeter features.

"Microfluidic" – by convention, refers to fluidic features having at least one critical dimension that is generally less than 500 micrometers. The narrowness of the critical dimension results in fundamental changes in the rules governing fluid flow. The liquid flow regime is characterized by Poiseuille or "laminar" flow.

"Stress" is the internal or restoring force per unit area associated with a strain and has units of Pascals or megaPascals.

"Strain" is a ratio $\Delta L/L_0$ of the change in length divided by the original length in response to an externally applied stress, and is unitless; it is often given in percent.

"Yield point" is the point on a stress-strain curve where the curve deflects or levels off and plastic deformation commences, and thus corresponds to the "elastic limit" of the material. Prior to the yield point, the material elastically return to its original shape when the applied stress is removed. Once the yield point is passed, some fraction of the deformation will be permanent and non-reversible. A yielded material, such as a diaphragm, has been stretched beyond its yield point.

"Yield Strength" and "yield point" are measured by standard techniques for reproducibility, such as described in ASTM Test Method D882-10 (the "882 test method"). For consistency, generally a 1 mil film is a preferred substrate. Yield strength is an indication of the maximum stress that can be developed in a material without causing irreversible deformation. Yield point is an indication of the maximum strain that can be developed in a material without causing irreversible deformation. For practical reasons, the measurements of yield strength, strain, elastic limit and elastic modulus are defined experimentally from a stress-strain diagram.

Offset yield strength is the stress read from the plot at the intersection of an offset line (drawn parallel to the initial slope of the stress-strain curve through the elastic deformation range) and the stress-strain curve, where the offset line is offset by a

selected value. Offsets for plastics are conventionally taken as 2%. Optionally, yield is sometimes shown as a range, for example in the case of co-laminated films.

"Elasticity" refers to the ability of a material to return to its original shape when load causing deformation is removed. Elasticity is the ability to store and release energy with a spring-like sample response generally as described by Hook's law of elasticity. If the strain increases linearly with increasing applied stress, the material is purely elastic, however in some materials, such as materials displaying viscoelastic properties, the stress-strain relation is not linear and the sample response is strongly dependent on time and rate of load application.

"Elastic modulus" (*E*), also termed "Elastic Modulus", is a slope measured in the elastic deformation region of the stress-strain curve, where strain is fully reversible. "Elastic Modulus" is the initial slope measured in the stress-strain curve and is an indication of the stiffness of the material. Elastic Modulus is a constant within the range of stretch or deformation that is fully reversible, and is thus equivalent to the spring constant of Hooke's Law.

"Permanent Deformation" or "inelastic deformation", is an increase in length dimension, expressed as a percentage of the original length dimension, by which material fails to return to its original length after being subjected to an elongation stress. When subjected to a stress greater than the yield strength or elastic limit of the film, permanent deformations of thin films may occur. For example, when a thin film diaphragm having a web span length from one side to another of a cavity or frame is stretched by a pressure and then collapsed back to a relaxed state, the web span length may be permanently elongated according to the amount of overstretch that the diaphragm was subjected to in excess of its yield point. "Overstretch" simply indicates that the material has been stretched past its yield point.

"Toughness" of a material is the ability of a material to absorb energy and plastically deform without fracturing or rupturing, and can be related to the total area under the stress-strain curve up to a break point according to the integral

$$K = \int_0^{\epsilon_f} \sigma \, d\epsilon$$

where ϵ is strain, ϵ_f is the strain on failure, and σ is stress. The units of K are of energy per unit volume. For purposes of the invention, toughness is particularly indicative of the capacity of a material to undergo a strain of up to 50% by length and to be permanently deformed thereby. This property is desirable for the manufacture of pneumatic elements by a form-in-place process as described herein.

A comparison of the relative magnitudes of the yield strength, ultimate tensile strength and percent elongation of different material can also give a good indication of their relative toughness.

"Top", "bottom", "up", "down", "upper", "lower", "above", "below", "upward", "downward", "superior to", "floor", "roof", "convex", "concave", and so forth, are indications of relative position and not absolute position or viewpoint: when reference is made to a specific frame of reference, such as the "ground plane", as taken orthogonally to an intersecting plumb line.

Unless the context requires otherwise, throughout the specification and claims that follow, the word "comprise" and variations thereof, such as, "comprises" and "comprising" are to be construed in an open, inclusive sense, that is as "including, but not limited to". Reference throughout this specification to "one embodiment", "an embodiment", "one aspect", or "an aspect" means that a particular feature, structure or characteristic described in connection with the embodiment or aspect may be included one embodiment but not necessarily all embodiments of the invention. Furthermore, the features, structures, or characteristics of the invention disclosed here may be combined in any suitable manner in one or more embodiments. "Conventional" is a term designating that which is known in the prior art to which this invention relates. "About" and "generally" are broadening expressions of inexactitude, describing a condition of being "more or less", "approximately", or "almost" in the sense of "just about", where variation would be insignificant, obvious, or of equivalent utility or function, and further indicating the existence of obvious minor exceptions to a norm, rule or limit.

Microfluidic Circuit Elements by a Yield-in-Place Process

Conventional art relies on diaphragms formed from elastomeric films or rigid sheets. However, we have found surprisingly that a hitherto unrealized class of diaphragm materials may be advantageously used in forming microvalves and micropumps. These diaphragm materials are polymers selected for toughness and chemical resistance, but substantially lack elasticity once stretched and are not excessively stiff. The unifying concept is a recognition that an overstretched material (e.g., yielded) selected for its toughness, having been stretched beyond its yield point so as to be deformed permanently, requires essentially no work to transition from a first state to a second state, each state conforming to an opposite aspect of a microcavity. The diaphragm is typically stretched before use or on first use and behaves as a flexible, flaccid, blister-shaped skin, inelastically controlling or propelling fluid flow according

to the pressure differential across the diaphragm. Once stretched, the diaphragm film does not return to its native dimensions, and advantageously, this results in decreased latency and increased stroke volume, decreased incidence of sticking of the diaphragm to the pump wall, and improved conveyance of bead slurries, for example.

FIGS. 1A and 1B show a plastic film diaphragm member being stretched by a form-in-place process of "over-stretching" the film beyond its yield point. In this case, a simplified pneumatic manifold 2500 and diaphragm 2501 is shown. The edge of the diaphragm is affixed to the substrate 2502 and an apron 2503 extends past the periphery of a pneumatic cavity 2504. The diaphragm itself forms a "web" over the cavity. During stretching under controlled process conditions, the diaphragm web covering the lower chamber is stretched and permanently deformed as shown in FIG. 1B. A fluid entering portal 2505 expands the diaphragm like a soap bubble, but upon release of pressure, instead of relaxing to its original sheet-like state, the diaphragm web will remain permanently stretched, forming a collapsible, flaccid, blister-shaped skin, and will have a larger stroke volume and decreased work function in transitioning from a distended state to a collapsed state. In contrast, an elastic material will resist distension and will recover to its native flatness when the pressure is removed.

For micropump and valve diaphragms formed by a yield-in-place process, materials having a yield point (when tested by ASTM D882 standard methods) in the range of about 2 to 30MPa are preferred, but materials having a yield point of about 100 or 120MPa may be used if desired. Diaphragm materials having yield points of 30MPa or less include, for example polyethylene, low density polyethylene, blends with high density polyethylene, polyolefin composites and laminates, polyvinylidene chloride composites and laminates, and ethylene vinyl acetate composites, while not limited thereto. It is well known that blends, block grafts and co-laminates (generally termed "composites") of these polymers may be formulated to tailor the yield strength for a particular application, for example a co-laminate consisting of low density polyethylene / ethylene vinyl acetate/ polyvinylidene chloride, / ethylene vinyl acetate, and low density polyethylene (LDPE/EVA/PVDC/EVA/LDPE) (as sold under the trade name SARANEX®) was found to have a yield strength of about 15MPa and has been demonstrated to be useful in the inventive micropumps and microvalves. Typically the films employed will have a thickness of 1 to 3 mils, although slightly thinner or thicker films may also be used. Other useful films will be apparent to one skilled in the art after study of this disclosure. One useful film having a yield point of about 100MPa is polyethylene terephthalate, which is readily available in a 1 mil

thickness in sheets or rolls, and which may be formed into stretched diaphragm webs of the invention by mechanical means as will be described below.

FIGS. 2A and 2B are views of a diaphragm film 2501 in a "distended state" and a "collapsed state". In the distended state (FIG. 2A) the film is essentially concave in shape, and in the collapsed shape (FIG. 2B), the film is generally amorphous and can be pressed flat. We have found, surprisingly, that the films function well as microvalve and micropump diaphragms. When pressed flat against a valve seat, for example, liquid flow through a valve is readily stopped under operating conditions typical for a microfluidic device. When operated as a pump, the pump stroke is essentially equal to the volume enclosed in the distended state less the volume enclosed when pressed flat by a pressure or a mechanical actuator, i.e., the micropump has essentially a zero deadspace volume.

In FIG. 2B, the amorphous web is collapsed but has not recovered elastically to its native flatness, and now has a surface area that is substantially greater than its virgin condition, having been overstretched past its yield point. The figure is drawn schematically and is not intended to represent the actual appearance of a distended or collapsed diaphragm member in use. Stretched diaphragm webs of this type may be formed with a surrounding apron 2503 as shown here, where the apron serves as lip to attach and seal the diaphragm to the substrate.

FIG. 3 is a schematic view defining the analytical geometry of mechanical strain in a diaphragm member adapted for a subcavity shaped as a spherical cap 2510. In this example, the cavity is defined by a span dimension or chordal length L from A to B and an internal height equal to the sagitta or height of the spherical cap segment. The diaphragm 2501 conforms to the interior surface of the subcavity 2510. Here the cap height is denoted by z, and is a fraction of the radius R. The internal wall of the subcavity from A to B defines an arc length L' of a sphere 2511 having radius R, where the arc is inscribed by center angle θ, and where O is the center of the sphere. Pump cavities of this kind can be made with a hemispherical milling head or by a molding process, for example. The arc length L' is then calculated from the central angle θ (in radians) and the radius R by the formulae $L' = \theta * R$ so that the overstretch can again be calculated as a permanent deformation L'/L. The permanent deformation, when expressed as a percentage, may be compared to a stress-strain curve for the material, where it will be seen that the deformation of the diaphragm webs of the invention exceeds the elastic limit of the material on the strain axis.

FIG. 4A is a cross-sectional view of a rectilinear pump microcavity 2520 formed by lamination, the laminate stack including a sheet of diaphragm material 2501.

In this example, the diaphragm is a continuous sheet and extends throughout the device body. The diaphragm layer separates the device into a lower body part and an upper body part. The lower body part includes hydraulic subcavity 2522, inlet 2523 and outlet 2524. The upper body part includes a pneumatic subcavity 2525 and pneumatic actuation port 2526. In this example, the two body parts are joined by a glue layer 2527. The structure may be assembled layer by layer, or may be assembled by first forming a lower body part, applying the diaphragm layer 2501 to the body part, and then building or adding an upper body part thereon. As shown here, the distended state "blister" may be formed by a yield-in-place process after the body has been assembled, generally by applying a pressure exceeding the yield point of the thin film material but not exceeding the mechanical strength of the cartridge body. Optionally, the blister may instead be formed before the final assembly is completed.

FIG. 4B is a schematic view defining the analytical geometry of mechanical strain of a diaphragm member adapted for a rectilinear cavity 2530. The cavity is characterized by a span dimension from A to B and an internal height h. Diaphragm 2531 (dashed lines) forms a "web" spanning and sealing the cavity. The dimensions are not necessarily constant on any axis, but for simplicity of explanation it can be seen that the internal surface of this rectilinear cavity is bounded by a length L' = 2h+L in this sectional view, where L is the length from A to B and h is the interior height of the cavity. When yielded under an applied pressure, the diaphragm web 2531 will stretch to generally conform to the internal dimensions of the cavity and will acquire a length $L' \approx 2h+L$, where the length L' is generally equal to two times the height of the chamber plus the web span length from side to side. The overstretch (L'>L) can then be quantitated and equals a permanent stretch deformation factor of L'/L. To achieve this, materials are selected so that the yield point (elastic limit) of the material is exceeded in the stretching process. Stretching is not necessarily uniform, and in some cases will be greatest along the peripheral border of the web.

FIG. 5A is a cutaway view of a pump structure 2540, depicting a microfluidic device with diaphragm 2541 in an overstretched configuration which partially fills the cavity. In FIG. 5B the diaphragm is stretched to more closely conform to the interior walls of the cavity. The diaphragm is advantageously and simply made by forming the pump structure to include a flat sheet of the selected diaphragm film sandwiched in place; then in a separate process step, the device is subjected to an internal pressure on one side of the diaphragm, where the pressure is configured to exceed the yield strength of the diaphragm film, thus irreversibly stretching the film. We term this a "yield-in-place" process. The diaphragm in its distended state after

stretching is seen to have a convex "blistered" or "ballooned" appearance that can be readily collapsed as earlier described with reference to FIG. 2B. Also shown in the figure is an "apron" 2542 used to seal the diaphragm between the pneumatic housing members (upper layers) and the fluidic housing members (lower layers). The apron in this example is contacted by a glue layer 2543 that bonds the fluidic face to the pneumatic face of the device. Also shown are pump inlet 2544 and outlet 2545, where directionality of fluid flow is established for example by the use of check valves (not shown) or other means known in the art. Also shown is pneumatic actuator port 2546 and pneumatic cavity 2547.

FIGS. 6A, 6B and 6C are a plan view and elevation views of a yield-in-place diaphragm member 2541 for a fluidic circuit element. In plan view, the apron 2542 is seen to be configured to seat in a generally cylindrical chamber. View 6B shows the appearance of the uncollapsed blister after its manufacturing process is completed *in situ*; however it will be understood that upon release of pressure, the web blister generally will collapse into an amorphous folded form and/or can be pressed so as to flatten against an opposing surface of the substrate, as drawn conceptually in FIG. 6C. The web has two states, a first distended state as shown in FIG. 6B and a second state as shown in FIG. 6C. However, it is understood that the film may be equally distended in both states, but at opposite sides of a microcavity, as would be the case if the microcavity consisted of hydraulic and pneumatic subcavities that had generally mirror symmetry. The two states may be described as being endpoints of a transitional process in which the diaphragm moves or inverts from the first state to the second state. In some instances, the pressure difference required to invert the film from a first state to a second state within the cavity is less than 3psi. In other instances, the pressure difference required to invert the film within the cavity is less than 1psi. In yet another working example, the pressure difference required to invert the film within the cavity is less than 0.1psi and is substantially less than the pressure difference required to overcome the inertia of the liquid in the hydraulic cavity.

FIG. 7 is an exploded view showing the construction of a pump structure 2540 by a process of lamination and includes a glue layer 2543. In this example, the blister shape of the diaphragm member 2541 is made after assembly by a "form in-place" process in which the yield strength of the diaphragm web is exceeded by an applied pressure. The diaphragm will be stretched to conform to the internal surface of the pneumatic cavity 2547. The diaphragm member has been cut to fit so that a single glue layer may be used.

FIG. 8 is an exploded view of a pump structure 2550 formed by lamination, where an uncut sheet of diaphragm material 2551 is layered in the assembly stack. Two glue layers (2552a, 2552b) bond the diaphragm layer in the stack. The blister shape is again formed after assembly by a process of applying an internal pressure on one side of the diaphragm. The diaphragm will be stretched to conform to the internal surface of the cavity.

FIGS. 9A and 9B demonstrate how a diaphragm-driven micropump 2560 can be formed by a yield-in-place process of yielding a plastic film. The difference in enclosed volume between the stretched diaphragm web (2561, FIG. 9B) and base 2563a of the hydraulic subcavity 2563 is the ejection stroke volume of the pump. At full stretch, the diaphragm web conforms proximately to the interior roof and walls 2562a of the pneumatic subcavity 2562. Thus the process of manufacturing mimics the process of pumping and ensures a consistent stroke volume. This was first noted with films that were not resilient elastomers but had been chosen for chemical resistance. A particular film in this class was a co-laminate of a polyethylene terephthalate or vinylidene chloride sandwich disposed between layers of polyethylene, which is valued for its chemical resistance. We found that the first ejection stroke volume of a virgin film was significantly less than second or third ejection stroke volume of the film under normal conditions of use. After investigation, it was discovered that the film had stretched past its yield point and was irreversibly deformed by the process. In use, the film is stretched so as to approximately conform and have the surface area of the interior wall surface 2562a of the pneumatic subcavity 2562. Also shown in the figure are the hydraulic cavity 2563, liquid inlet 2564, liquid outlet 2565, glue layer 2566, molded substrate layer 2567, and pneumatic actuation duct 2568.

The form-in-place process is advantageous in its simplicity, but other means for forming an overstretched diaphragm include use of male and/or female molds to form the "blister" features on the sheet prior to assembly, where a sheet having pre-formed blisters is mated to align the locally-stretched diaphragm features with pre-formed cavities in the cartridge body. Pre-stretching of the diaphragms may be done with a mechanical platen press, or can involve a roll-to-roll process using a rotating die. Vacuum forming of the stretched web elements is also conceived.

In another variant of the process, a sheet of a thin film material may be layered over a pre-formed body half having cavities and circuit features. A press or a soft roller may then be used to stretch the film into the cavities, and a second body half may then be mated with the first to sandwich the diaphragm features in place. Excess material may be removed if desired. In some instances the diaphragm and the cartridge

body are made of like or similar materials and can be bonded by thermal, ultrasonic or solvent welding. In other instances glue is used.

FIGS. 9A and 9B also demonstrate a comparison of thin film diaphragm 2561 in a virgin versus an overstretched configuration. In the overstretched configuration, the distended web of film 2561 has an arcuate length measured along its surface from edge to edge of the cavity 2505 that is longer than the chordal length (as measured along the unstretched film). The surface area of the stretched web member approximates the surface area of the internal roof and walls of the pneumatic subcavity 2562 (or will have the surface area of the larger of the two subcavities when stretched to fit). The enclosed volume of the stretched state is greater than the collapsed or relaxed state. Thus operating the device by use of pneumatic pressure at pressure port 2568 can be used to drive the diaphragm from a distended to a collapsed state in alternation, thus achieving a pump stroke for filling and ejecting fluid from subcavity 2563. These pumps are self-priming.

Stroke volume maturation is shown in FIGS. 10A and 10B. Ejection stroke volume of a stretchable plastic film before (PRE) and post (POST) stretch past the yield point is shown to result in a gain in stroke volume. It was discovered that diaphragm webs were stretching after assembly because the stroke volume increased with repeated use; this phenomenon was then exploited to form pre-stretched diaphragms having a maximal stroke volume for the dimensions of the pump cavity.

As a matter of quality control and reproducibility of operation, it proved advantageous to perform this stretching process prior to release of product or to conduct a "warm up" operation prior to starting an assay. Once the stretching process is complete, the stretched diaphragms operate with an increased ejection stroke volume (and decreased response time) that is no longer dampened by the elasticity of the film, as had been problematic with pumps and valves of the prior art. Materials may be used that are tougher and more chemically resistant than the polyurethane rubber diaphragms of the prior art. Typically these materials have yield points under 30MPa, and more preferably under 20MPa for micro-dimensioned fluidic features, but the blister may also be formed using mechanical means as described below, thus allowing those skilled in the art to form the inventive yielded webs from materials having higher yield strengths and correspondingly higher elastic moduli. Once stretched, the resistance required to transition the diaphragm web from a distended to a flaccidly collapsed state is negligible, such that the work required to move the film is essentially only that needed to overcome inertia of a fluid in the chamber, with no added work required to overcome the restorative force of the elastomeric diaphragms of the prior art.

As shown in FIG. 10A, ejection stroke volume for a SARANEX® diaphragm having a diameter of about 1.08 cm was found to increase from about 90 microliters (PRE) to 150 microliters (POST) by becoming overstretched, more than a 50% increase. The cavity ceiling (FIG. 9B, 320) limits ultimate stretch dimensions of the film and ensures a higher level of consistency of the nominal stroke volume in the manufactured product.

Similarly, as shown in FIG. 10B, a diaphragm having a diameter of about 0.88 cm was found have an ejection stroke volume of 50 microliters (PRE) before stretching and about 90 microliters (POST) after stretching, about an 80% increase. Diaphragms which have been stretched may be stored in a collapsed state until needed.

To better understand the materials behavior underlying the results shown in FIG. 10, we performed stress-strain analyses of several representative films. Yield stress and yield strain (load and deflection) were measured on an Instron universal materials testing machine (Instron, Norwood, MA) operated at strain ramping speed of 150 millimeters/min, a gauge length of 15 cm between grips, and a grip width of about 2.2 cm. Samples of films were generally 1 mil in thickness unless otherwise noted. Yield strengths were determined by standard methods as outlined in ASTM Test Protocol D882 (100 Barr Harbor Dr., PO Box C700, West Conshohocken, PA 19428). Testing is generally done at a controlled room temperature of about 23 °C. Measured yield strengths are dependent on strain rate, temperature, and film characteristics, and one skilled in the art will understand that the material property parameters cited here are given with reference to standardized testing conditions.

One material of interest is low density polyethylene / ethylene vinyl acetate/ polyvinylidene chloride, / ethylene vinyl acetate, and low density polyethylene (LDPE/EVA/PVDC/EVA/LDPE) co-laminate, sold under the trade name SARANEX®. FIG. 11 is a stress-strain analysis for a PVDC/PET/PE co-laminate film. The material has a limited elastic modulus and begins to deform at a yield point in the range of 12 – 16MPa. The slope over the reversibly elastic range corresponding to an elastic modulus E = ~80MPa. At 50% on the strain axis, the material was allowed to relax, but displays an intermediate level of hysteresis with a return to zero stress, indicating a permanent deformation of 30% (*i.e.*, L'/L). This overstretch results in an essentially stress-less response in subsequent cycles, as shown below.

FIG. 12 depicts a stress-strain curve obtained with a 1 mil film of Polyurethane 7010 elastomer (Deerfield Urethane Inc., South Deerfield, MA). The film demonstrates elastic behavior over a broad strain range. No identifiable yield point is noted in this range and the minimal hysteresis on contraction is consistent with its

spring-like behavior. Similar results were obtained with a second elastomeric polyurethane sample.

Disadvantageously, polyurethane 7010 was found to sweat or crack when exposed to common solvents used in biochemical assays, particularly solvents such as ethanol and methanol, or chaeotropes such as guanidinium salts. The diaphragm material degrades within minutes, pneumatic integrity is impaired, and the diaphragm can cease to function as a seal between the pneumatic and hydraulic subcavities. This behavior renders use of these polyurethanes problematic in certain molecular biological assay cartridges.

FIG. 13 is a stress-strain curve obtained with a thin film of biaxially oriented polyethylene terephthalate (BoPET, sold under the trade name MYLAR® by DuPont Teijin Films, Hopewell VA). The material is very stiff, as indicated by the sharply elevated elastic modulus and the yield point above 100MPa. The elastic limit of the material is exceeded at about 5% deformation. However, the lack of deflection under operating pressures likely to be useful in real-world devices also raises the issue as to whether a device having these materials can be reliably self-priming. While BoPET is expected to be a tough material, it is a high modulus material and has a stiffness that is problematic in a yield-in-place process within the plastic body of a cartridge.

FIG. 14 is a stress strain curve for a PVDC/PET/PE co-laminate taken after repeated cycles of strain and relaxation. As can be seen, following the first cycle (FIG. 11), subsequent cycles display little or no "memory" of the virgin film properties and shape. The slightest pressure results in a rapid ballooning of the film and essentially no elastic recovery is observed. This behavior reduces stroke response time and resistance compared to elastomeric materials. In its stretched form, the film retains a suitable level of toughness for use in disposable cartridges of the invention.

FIG. 15 shows the effect of repeated cycles of strain and relaxation on BoPET. Once plastic deformation is achieved, the overstretched material loses any resistance to serial application of pressure. On repeated exercise of the film, essentially no force is necessary to distend the film (dashed line). However, the initial stretch step (solid line) requires a substantial application of force.

The size of features that can be formed in a material having a defined yield strength is dependent on the applied pressure or force such that an increased degree of miniaturization that can be achieved by selecting materials with lower yield strengths. However, materials that have very low yield strengths, such as those having a yield strength less than 2MPa, are likely to prove delicate and difficult to handle in a

manufacturing environment and for that reason are not considered to be good candidates for making the locally stretched diaphragm webs of the invention.

FIGS. 16A and 16B are cross-sectional views of a microvalve 2600 in the body of a microfluidic device, showing an "ON" (or "open") and an "OFF" (or "closed") configuration of a diaphragm web 2601. The valve body is formed of multiple layers which include outside capping layer 2602 and a core formed by fusion of a molded pneumatic plate member 2603, a via plate member 2604, and a molded hydraulic plate member 2605 such as by diffusion bonding, or by an optional ACA glue layer 2612. Diaphragm 2601 is sandwiched between plates 2603 and 2604. The valve cavity consists of a pneumatic cavity 2606 and a hydraulic cavity 2607 separated by the diaphragm. Two fluidic channels (2608, 2609) are shown entering the hydraulic cavity 2607 through dual ports in a valve seat; the ports are separated by a valve sill 2610. In the closed position (FIG. 16B), the valve diaphragm seats on the valve sill and is pressurized at pneumatic port 2611 to resist flow of fluid from one channel to another. In the open position (FIG. 16A), the diaphragm is retracted into the pneumatic cavity 2808 and fluid is free to flow across the valve sill from channel 2608 to 2609. Movements of the diaphragm are actuated by a pneumatic subcircuit ported into the pneumatic cavity at port 2611.

In other words, the valve includes a) a plastic body with internal valve cavity, the valve cavity being defined by a first enclosing lower surface and a second enclosing upper surface, where the first surface defines a valve seat and the second surface sealingly apposes the first surface at a lip 2620 bounding the valve cavity; b) a diaphragm member with apron 2621 peripherally defined therearound, wherein the apron is sealedly inserted into the body under the lip to separate the first surface from the second surface; c) a first fluidic channel entering the valve cavity through the valve seat at a first port; d) a second fluidic channel entering the valve cavity through the valve seat at a second port; e) a valve sill 2810 defined on the first surface between the first port and the second port; and further wherein the diaphragm member is capable of being reversibly deflected against and retracted from the valve sill, thereby defining an "OPEN" position and an "OFF" position for allowing or not allowing flow of a fluid between the first channel and the second channel.

FIG. 17 is a cutaway perspective view of the valve structure of FIG. 16. In this instance the footprint of the valve has a roughly "peanut" shape with an obvious waist narrowing in proximity to the valve sill 2610. The diaphragm is shown as a partially distended, half peanut-shaped bulb within the valve cavity.

The peanut shape can be seen more clearly in FIG. 18A. The diaphragm member 2601 is bilobate and includes an apron 2621 peripherally disposed around the central bulbs. Positions of the inlet and outlet ports (which are not part of the diaphragm) are indicated as dotted lines (2608a, 2609a). FIG. 18B is an elevation/perspective view of a diaphragm member 2601 with apron 2621 for a fluidic microvalve. The surface area of the stretched web approximates the interior wall surface area of the pneumatic subcavity 2606. The valve diaphragm is stretched into a bulbous or "blister" shape (conforming generally to the internal cavity in which it is formed) after depressurization and, unlike valves formed with an elastomeric diaphragm, may be advantageously supplied in the "OPEN" position in which there is essentially no resistance to fluid flow in the hydraulics of the device (i.e., from channel 2608 to 2609). Application of positive pressure through the pneumatic control line 2611 collapses the diaphragm against the valve seat 2610 and turns the valve "OFF".

FIG. 19 is an exploded view of the valve device 2600 of FIGS. 16-18. By stretching a suitable diaphragm material, the footprint of the valve body can be further miniaturized. Because of the pliancy of LDPE/EVA/PVDC/EVA/LDPE and other stretch wrap films, for example, very small diaphragm features can be prestressed using the yield-in-place process. Shown are stretched diaphragm 2601 with peripheral apron 2621, capping layer 2602, pneumatic layer 2603 with pneumatic cavity 2606, via layer 2604, hydraulic layer 2605, with inlet and outlet channels (2608, 2609) as marked.

The depth of the valve cavity 2606 in the z-dimension is exaggerated for purposes of illustration. Valves of this type may be manufactured in the "OPEN" position, but can be reversibly closed at high speed (*i.e.*, with reduced latency) by applying a pneumatic pressure of 2 – 12psi through the pneumatic control line 2611. The valve diaphragm is stretched into a three-dimensional blister shape by application of pressure during manufacture, as in a yield-in-place process described with reference to FIG. 5, or by mechanical action as part of assembly or pre-assembly. In the yield-in-place process, pressure is applied through the fluidic circuit or by applying negative pressure through the pneumatic circuit so as to yield and conform the polymeric film to the bulbous interior contour of the pneumatic subcavity 2606. Alternate mechanical processes for pre-stretching the web will be described below.

By selection of a suitable film, and by adjustment of the conditions for yield-in-place stretching of the film during the manufacturing process, valves of this type having valve seats of less than about 0.5mm in length and 0.3mm in width are readily obtained. Referring to the yield-in-place process, preferred films for millimeter-sized valves include linear low density polyethylene (particularly metallocene-catalyzed

LLDPE), low density polyethylene blends and co-laminates generally, polyethylene vinyl acetate copolymers and laminates, polyvinylidene chloride and PVDC co-polymer and laminates, and selected polyolefin composites and laminates, while not limited thereto. With a suitable film, pneumatic valve features in the sub-millimeter scale are approachable. Generally, films having yield strengths of less than 15 or 20MPa under manufacturing conditions are preferable for making smaller valve features. A particularly preferred range is 5 to 20MPa; and for some applications 2 to 15MPa. While yield strengths are cited for films under standard test conditions, it is understood that increased process temperature may also be used to optimize conditions for manufacture of microvalves by this method.

As shown in FIG. 20, alternate valve structures 2800 may be formed having yielded diaphragm webs. The process of stretching the web to conform to an internal surface of the valve cavity may be used to make zero deadspace valves that are fluidically "OPEN" or are fluidically "OFF" when shipped. Initial stretching is generally achieved by applying a positive pressure or force to the top of the diaphragm, forcing the diaphragm against the bottom surface of the bowl that forms the valve seat or land. The valve is shown in the "OFF" configuration in FIG. 20B. However, application of a pressure pulse to the fluid side (and optionally zero or suction pressure on the pneumatic side), readily allows the valve to open because there is essentially no elastic modulus-related resistance as in prior art valves. Alternatively, a suction pressure can be applied to the pneumatic cavity, which transitions the valve back to the state or configuration shown in FIG. 20A, but with the stretched diaphragm having a collapsed blister shape in which the surface area of the valve is greater than the upper internal surface area of the valve chamber, thus resulting in a folded irregular condition of the web which is readily reversible in the closed, distended state. In our experience, these valves can withstand many actuations and closures during a typical cartridge lifetime, which is a few minutes to a few hours, and the deformation is not a hindrance to fluid flow in the "OPEN" state. Diaphragm materials in these cartridges are generally yielded to form permanently overstretched film structures before release for sale, but may also be yielded in some embodiments during a preparatory step immediately before use in an assay.

In use, these valves may also be closed by applying a suction pressure to a downstream fluid column or by applying a positive pressure to the pneumatics, thus draining and/or expelling any residual fluid volume from the valve cavity. Control of gas (venting) or fluid flow through the valve may be modulated by varying the ratio of

pressure on the hydraulic and pneumatic sides of the diaphragm or by applying pulsatile waveforms of alternating positive and negative pressures.

The valves may be constructed by lamination or by fusion of molded body parts. Shown here are top capping layer 2802, diaphragm 2801, pneumatic body layer 2803, hydraulic body layer 2804, and bottom capping layer 2805. Also shown are valve seat 2807, pneumatic cavity 2808, hydraulic cavity 2809, first fluidic channel 2810, second fluidic channel 2811 and pneumatic actuation circuit 2812. The dark arrow indicates fluid flow when the valve is in the "OPEN" position (FIG. 20A). The double arrow indicates transition from the "OPEN" position to the "OFF" position, where fluid flow is blocked by the distended diaphragm on the valve seat 2807 (FIG. 20B).

The hydraulic body part 2803 and pneumatic body part 2804 are depicted as molded parts and are joined at the dashed line by the diaphragm layer 2801, which may be a layer of BoPET, SARANEX, polyvinylidene chloride, or other thin film that is stretchable by the processes described here. Optionally, elastic thin films may be used. However, a pre-stretched film is advantageously flaccid and is actuated without the inherent resistance of an elastomer. Hydraulic pressure in a liquid entering the valve from port 2810 is sufficient to cause fluid flow in a fully open state.

FIG. 21 is a view of a yielded, bilobately stretched diaphragm or "blister" diaphragm of a microdevice 2800, and the web of the diaphragm is surrounded by an apron 2815. The apron is pinched between the body layers (2803, 2804) around the edges to separate the hydraulic subcavity and the pneumatic subcavity (2808, 2809) of the microvalve. This blister shape is flaccid, and the work required to collapse the blister is negligible relative to the work required to overcome the inertia of the fluid under microfluidic conditions.

FIGS. 22A and 22B introduce the concept of valve latency. The time it takes to turn on and off a valve is important in all fluidic processes, particularly for diagnostic assays where mixing, heating, and reaction kinetics are dependent on rapid valve actuation. In this example, a 10psi sustained pressure on the pneumatic side of the diaphragm is responsible for keeping the valve in the CLOSED state at the START of the timeline where the timeline is represented by arrow 2820 running from left to right. By releasing the positive pressure and applying a negative 5psi suction pressure on the pneumatic side of the diaphragm at time 2821, the valve begins to transition to the OPEN state. However, this transition is not instantaneous and the delay is termed the "latency time". In addition to the obvious concerns about stickiness of the diaphragm against the valve land, there are also material stiffness and elasticity that can

slow valve opening. Thus as achieved by conventional arts, valve latencies can be about 100ms. However, and as shown in FIG. 22B, by pre-treating the diaphragm by a stretching process, valve latency is substantially reduced. Because the material is pre-stretched, its resistance to transitioning from an open valve state to a closed valve state is negligible, and can occur with minimal pressure, either in response to a negative pressure in the pneumatic actuation manifold, or by a positive fluid pressure in the hydraulic works. Latency in the example shown, using a SARANEX® diaphragm was measured at about 100ms prior to stretching but after pre-stretching (POST) was reduced to less than 20ms to full opening. This is a surprising result and would not have been an obvious action of known elements behaving in predictable ways.

A negative pressure in the hydraulic works or a positive pressure in the pneumatic works is generally sufficient to close the valve depicted in FIG. 20, for example. Moreover, these valves may advantageously be used in valve logic trees having a zero pressure state (typically a vent to atmosphere on the pneumatic side). In short, the valves may be operated to open passively and close actively, if desired, an advance in the art over conventional valves. Surprisingly, this innovation eliminates the problem of sticky valves that can occur in stored product using elastomeric or rigid diaphragm materials.

Thus in another aspect, a pneumohydraulic valve is provided wherein the valve is OPEN with no resistance to hydrostatically driven fluid flow when said pneumatic cavity is at atmospheric pressure (*i.e.*, the pre-stretched diaphragm is flaccid) and OFF or closed when pressurized by a pneumatic pressure greater than a hydrostatic pressure in the hydraulic chamber (for example as shown in FIG. 20, the pre-stretched valve conforming to the features of the valve seat 2807 to fluidly separate the inlet and outlet ports). More broadly, in this aspect, the invention comprises a pneumohydraulic valve having a pneumatic cavity and a hydraulic cavity separated by a yielded diaphragm film, wherein the valve is OPEN with no resistance to hydrostatically driven fluid flow when said pneumatic cavity is at atmospheric pressure and CLOSED or off when pressurized by a pneumatic pressure greater than a hydrostatic pressure at either the inlet or the outlet of the hydraulic chamber. It would be understood by those skilled in the art, that increasing hydrostatic pressure on a liquid in the hydraulic cavity would readily displace a flaccid diaphragm film, and conversely, increasing the pneumatic pressure relative to the hydraulic pressure would inflate the diaphragm to its stretched shape and displace any fluid from the hydraulic cavity.

We now introduce the concept of a critical web dimension L_c as theory, where the "web" of a diaphragm 2501 is modeled as a circular pressurized bulge (as

shown pictographically in FIG. 1A and 2A) and chordal length L is defined in reference to FIG. 3. The critical web dimension L_c is the minimal web length for which a defined pressure will result in a defined overstretch. The smaller the web dimension, the more pressure required to achieve the desired stretch. The relationship approximates a parabolic curve in which a pressure in excess of 30psi is needed to achieve deformation of a 2mm web of a thin film having selected material properties. The derivation generally follows Freund LB and S Suresh, 2004. *Film buckling, bulging and peeling.* In, Thin film materials – Stress Defect Formation and Surface Evolution. Cambridge University Press, pp 312-386. A circular web of a thin film was analyzed as it deforms into a spherical cap by the action of an applied pressure P. Assuming the thin film deforms with a constant radius (*i.e.*, a spherical deformation) and a constant equi-biaxial strain throughout the membrane (*i.e.*, the radial and circumferential strains are equal and constant at all points in the film), the stress in the membrane can then be related to strain as a function of Young's modulus, Poisson's ratio, and the film thickness. Neglecting bending stress within the material (*i.e.*, assuming a thin film), the stress at the circumference of the feature can be related to the pressure. Strain is then calculated as L'/L, the change in arc length from a flat film to a distended film. Finally, the critical diameter L_c for a given pressure can be derived analytically in cases where the deflection of the membrane is much less than the diameter of the feature.

In FIG. 23, the critical web dimension L_c of a film having the characteristics of SARANEX® film is calculated and plotted against the pressure at which a 6% yield is expected to occur. As can be seen, as the film dimension L is reduced, higher pressure is required to stretch the film. Plastic deformation of SARANEX® of more than 6.0% is predicted only if the diaphragm web spans a dimension greater than L_c for a given pressure. For example, a yield-in-place feature of about 3mm is expected by stretching a SARANEX® diaphragm under 30psi of applied pressure, which approaches the internal pressure at which damage to a laminated microfluidic cartridge is expected.

However, the above analysis has several shortcomings: the assumption of a spherical deflection and equi-biaxial strain are not valid for non-circular geometry, and strain will not be constant throughout the web in a real valve. Also, the assumption that the deflection is significantly less than the feature size does not hold, which will lead to an inaccurate analytical result. Another shortcoming is the insufficiency of the material data for Young's modulus, which is rate dependent, and Poisson's ratio. The strain rate used in standardized materials testing is likely at least an order of magnitude less than that of a pneumatically actuated valve in operation. Lastly, as the film

distends and thins, the stress for a given pressure will be higher due to a reduced cross sectional area. This could push the estimate of L_c to higher pressures or larger diameters. In short, the engineering is highly unpredictable and complex, and the behavior of working valves cannot realistically be achieved without experiment.

Surprisingly, in spite of theory, we found experimentally, using pressures less than 20psi, that valve diaphragms may be formed from 1 mil SARANEX® thin films (having a yield point in the range of 12 – 14MPa) in cavities having dimensions of about 2 x 3mm and geometry essentially as shown in FIG. 18. This finding was unexpected, but welcome, because 20psi (at elevated temperature) is within the working limit for pressurization of laminated device bodies without damage. Overstretch achieved was greater than 6% by length. Our theoretical analysis had suggested that microvalves of this size could not successfully be made at pressures less than 30psi, raising the concern that "yield-in-place" or "form-in-place" stretch processing in a laminated device body would be impractical because of limitations on internal pressure that could be tolerated by the device.

FIG. 24 shows the results of an experimental study of overstretch behavior of a LDPE/EVA/PVDC/EVA/LDPE co-laminate diaphragm sealed over a pneumatic chamber that is about 2mm by 3mm. An initial pressure was applied to remove any initial looseness. Center point deflection was measured optically before and after application of a pressure sufficient to cause plastic deformation of the film. The change in plastic strain $\Delta\epsilon$ is calculated as

$$\Delta\epsilon = \sqrt{(\delta_5^2 + R^2)} - \sqrt{(\delta_0^2 + R^2))} / \sqrt{(\delta_0^2 + R^2)}$$

where load deflections are measured optically before (δ_0) and after (δ_5) pressure treatment of the film. Three results (as increase in centerline stroke displacement) are shown following pressure treatment at 10, 15 and 18psi. An approximate doubling of the membrane stretch was obtained after pre-treatment at 18psi, significantly greater than at 10psi, demonstrating that treatment with about 15 - 20psi is sufficient for yield-in-place diaphragm manufacture using LDPE/EVA/PVDC/EVA/LDPE co-laminate and the valve cavity has a height above the valve seat of about 100 micrometers.

This demonstrates that the inventive process achieves surprisingly small microvalve features, features having dimensions that would not have been predictably achieved by application of known methods. For thin films having a yield strength of less than 30MPa, standardized process pressure and temperature conditions can be selected to achieve stretch-in-place fluidic features having a desired size range. And as a corollary to that finding, using films having yield strengths less than 15MPa will

result in yet smaller pump and valve features, an improvement that enables increased miniaturization. A synergy is achieved by using materials having lower yield points as valve diaphragms, and stronger materials for structural members forming the microvalve cavity and associated channels.

Although PET is not considered suitable as a thin film for manufacture of form-in-place microvalves due to its high yield stress, PET is useful for the manufacture of device bodies, and thin films of PET can be incorporated by mechanically stretching the material where diaphragm webs are to be formed. Thus the invention is not limited to lower strength diaphragm materials. Where mechanical stretch processing is contemplated, a broader range of yield strengths may be considered. For example, while not limited thereto, materials such as BoPET, having a yield strength of about 100MPa, may be incorporated by a mechanical stretch process as described in the following figures.

FIGS. 25A through 25F are sequential views of steps in a first process of mechanically stretching a diaphragm film in place. The figures describe a process of building a microfluidic device enclosing a valve and a pump cavity, where both diaphragms are stretched by a mechanic press prior to final assembly. FIG. 25A shows a first molded body member 2900 having internal cavities and channels; and in FIG. 25B a lower capping layer 2901 is added to seal the channels. Viewed in cross-section are a hydraulic subcavity of a valve 2902 (with valve sill separating two fluidic channels) and a hydraulic subcavity of a pump 2903 (with an inlet and an outlet). This forms the hydraulic works of the device, and defines a hydraulic valve subcavity and pump subcavity with internal pumping as depicted schematically. In FIG. 25C, a thin film sheet of a suitable diaphragm film 2904 is overlaid on top of the hydraulic subassembly, covering the valve and pump subcavities. Then, the thin film material is welded or tacked to the molded part and excess material is stripped away in a laser cut-weld, resulting in the valve and pump diaphragm web members (2912, 2913) depicted in FIG. 25D. The welding and cutting step is performed using a laser on an X-Y table, for example. In a next step (FIGS. 25E to 25F), mechanical fingers (2910, 2911) having suitable dimensions are used to force (arrows) the diaphragm webs into the hydraulic subcavities under process conditions suitable to overstretch the film past its yield point and permanently deform the webs into the shape of a blister. Here the completed prestretched diaphragm web blisters (2915, 2916) corresponding to a stretched valve diaphragm web and a pump diaphragm web respectively, are shown in a distended state conforming to the shape of the internal surface area of the hydraulic cavities. A roller of suitable durometer could also be used to perform this process step.

Upper layers of the device may then be glued or otherwise fused or affixed to the hydraulic subassembly, sandwiching the diaphragm member in the completed device body. Final assembly is not shown, but would be understood by one skilled in the art and in reference to FIG. 7 and FIG. 19, for example.

FIGS. 26A through 26F are sequential views of steps in a second process of mechanically stretching a diaphragm film in place. FIG. 26A shows a first molded body member 2900 having internal cavities and channels; and in FIG. 26B a lower capping layer 2901 is added to seal the channels. Viewed in cross-section are a hydraulic subcavity of a valve 2902 (with valve sill separating two fluidic channels) and a hydraulic subcavity of a pump 2903 (with an inlet and an outlet). This forms the hydraulic works of the device, and defines a hydraulic valve subcavity and pump subcavity with internal pumping as depicted schematically. In FIG. 26C, a thin film sheet of a suitable diaphragm film 2904 is overlaid on top of the hydraulic subassembly, covering the valve and pump subcavities. Then, the thin film material 2904 is welded or tacked to the molded part and excess material is stripped away in a laser cut-weld, resulting in the valve and pump diaphragm members (2912, 2913) depicted in FIG. 26D. In this example, the cutting and fusion process is performed with an annular knife 2905 (or other suitable shape). Optionally the knife is heated or is sonically actuated so as to fuse the apron of the diaphragm members to the substrate. In a next step (FIGS. 26E to 26F), mechanical fingers (2910, 2911) having suitable dimensions are used to force (arrows) the diaphragm webs into the hydraulic subcavities under process conditions suitable to overstretch the film past its yield point and permanently deform the webs into the shape of a blister. Here the completed prestretched diaphragm web blisters (2915, 2916) corresponding to a valve diaphragm web and a pump diaphragm web respectively, are shown in a distended state conforming to the shape of the internal surface area of the hydraulic cavities. A roller of suitable durometer could also be used to perform this process step. Upper layers of the device may then be glued or affixed to the hydraulic subassembly, sandwiching the diaphragm member in the completed device body.

The teaching of the invention is not limited to valves and pumps, but relates to stretched diaphragms having a variety of functions in microfluidic devices. In the next example, we show a vent having a microporous diaphragm, the vent occupying the terminus of a channel. By selecting a hydrophobic microporous film, liquid may enter the hydraulic cavity while air is vented, allowing the cavity to fully fill with liquid.

Venting Elements having Breathable Diaphragms

FIGS. 27A and 27B are representations of a close-ended micropump cavity 3000 which finds use in a fluidic circuit having a branch that terminates in a chamber 3004 with no outlet. Fluid enters the chamber shown here through an inlet 3002 and fills the hydraulic subcavity 3004. In chambers of this type having diaphragms of the prior art, the resident gas cannot be displaced. However, by supplying a diaphragm web 3001 of a breathable microporous film, gas is voided during the liquid filling process through the diaphragm, through pneumatic subcavity 3005 and out port 3006. By "breathable" is meant a film having permeability to gas but not to liquid. The diaphragm may then be pneumatically actuated to expel the fluid from the chamber, because the diaphragm, once wetted, no longer vents gas robustly but instead is transformed into an efficient pumping member when actuated pneumatically. This novel feature combines a microporous vent and a pump into an integral unit with a single diaphragm. Port 3006 serves as a vent to outside atmosphere during the fill cycle, but may be valved so as to be operated by positive or negative pressure when operating diaphragm 3001 as a pump. Inlet port 3002 also serves as the liquid outlet.

In application, a series of chambers in a fluidic device are fluidly connected to perform an assay, the end-terminal chamber (FIG. 27) of the series having a breathable diaphragm 3001 that serves as a vent, so that any air trapped in the dead end chamber may be removed during the fill operation. By pressurizing pneumatic cavity 3005, liquid is expelled back out the inlet 3002 and returned to the upstream fluidic circuit.

Micropumps of this kind can also be used for reagent additions where a dried reagent is stored in the chamber for wetting at time of use, and for thermocycling, for example, where a pair of pumps are slaved so that one is actuated pneumatically, and the second is a close-ended fluidic branch having a terminal chamber that is filled under pressure (while venting gas through a microporous diaphragm) and then can be operated as a pneumatic pump to eject the liquid when acted on by a pneumatic overpressure.

FIG. 28 depicts construction of a vented micropump element by lamination. The material may be pre-stretched by applying a pressure differential across its interface as described with reference to FIGS. 4 and 5 or by using the mechanical stretch/assembly processes illustrated in FIGS. 25 and 26.

In another application, as shown in the device of FIG. 29A, breathable microporous films 3021 that have been stretched may be used to form a flow-through degassing 3020 chamber. This chamber includes an inlet 3022 and an outlet 3023 for

enabling flow of a liquid, but is topped by a pneumatic subchamber separated from the hydraulics by a microporous membrane 3021 (dashed line) through which gas can be withdrawn through port 3024 under a pressure differential. Films formed in this way can be used for degassing small volumes of fluids. Breathable microporous films can also be used in chambers 3030 configured to add gas as shown in FIG. 29B, such as for oxygenation of cells in flow cytometric applications or for introduction of bubbles through thin film 3031 (dashed line) into a fluid channel as where it is desirable to reform flow into a stream of alternating fluid and bubble segments, as was achieved at a macroscopic scale in the AutoAnalyzer II, where bubbles were introduced at a "tee" into tubing carrying assay fluids at regular intervals to disrupt the unstirred boundary layer as is problematic in some microfluidic applications. These films may be pre-stretched by the teachings of the invention to reduce resistance to fluid flow and to increase surface area for gas exchange.

FIGS. 30A, B and C are electron micrographs of the fine structure of a breathable microporous polyurethane film, such as is useful in the devices of FIG. 28 and 29. A porous, fractured cellular structure is readily visible with increasing magnification by scanning electron microscopy. Microporous polyurethanes include films sold as "PORELLE®" membranes (PIL Membranes Ltd, Kings Lynn, Norfolk UK). These polyurethanes can preferably be hydrophobic, but hydrophilic films may also be useful. One example is Porelle 355.

Other microporous polymers are also known and function analogously. Microporous forms of polytetrafluoroethylene (PTFE) sold under the trade name MUPOR® (Porex, Fairburn GA) are readily yielded in place using hydraulic pressure. A yield point of 2.2MPa was determined experimentally for a 1 mil PFTE film under standard ASTM test conditions. The resulting diaphragms have good permeability to gas and can be used as vents, and the hydrophobicity results in selective blockage of aqueous liquids if desired. In an unexpected solution to a technical problem, microporous polyurethane films may thus be used to form diaphragm members in closed-end channels, where ingress of liquid into a terminal chamber is possible only by directly venting the resident air through a permeable diaphragm. In some applications, these diaphragms initially release air, but when wetted, permeability to air is substantially decreased, thus the diaphragm to a zero-air entrapment, self-priming pump for close-ended channels, where advantageously the pump becomes an active pneumatic micropump once all air in the line is vented and the film is wetted.

Application of dry pneumatic pressure or hydraulic pressure is sufficient to cause films having a yield strength in the range of 2 – 30MPa to yield. The films will

generally conform to the shape of an internal cavity. Suitable form-in-place processes ensure that the full volume of the cavity is available for a subsequent pump stroke in the presence of liquid, and is useful for example, when pump chambers are used in pairs, such as for two-zone thermocycling, particularly when one of the pump chambers is a terminal chamber and is not otherwise vented. Alternatively, mechanical yielding processes of the invention may be used to form these yielded diaphragm members.

Circuit Combinations of the Inventive Fluidic Diaphragm Elements

FIG. 31 illustrates a representative fluidic circuit 3040 having a combination of diaphragm-operated circuit elements of the invention, as shown by drawing the channels and chambers without supporting substrate. This fluidic circuit consists of an inlet port 3041, a degassing chamber 3042 connected to a sanitary vent 3043, two paired diaphragm pumps (3044,3045) for back-and-forth fluid flow, a detection chamber 3046 with optical windows on the long axis and a terminal vent 3047. In operation, sample is added with aspiration, and is then directed through the circuit by coordinated action of pneumatic actuators on diaphragms within each circuit element. In an assay, dried reagents (circles) prepositioned in the hydraulic subcavities result in chemical reactions leading to a detection endpoint. Also needed for operation are two valves (3048, 3049) that are used to fluidically isolate the paired diaphragm pumps during reciprocating flow as in PCR amplification, where one diaphragm pump is held at an annealing temperature and the other diaphragm pump at a denaturing temperature, as is known in the art. Advantageously, pre-stretched diaphragm members achieve improved pump stroke volumes, improved consistency of stroke volume, reduce the work of pumping, and also improve the speed of the valves. Use of a stretched microporous diaphragm for venting bubbles in the liquid is also contemplated and has been shown to offer advantages when filling close-ended channels. These circuit combinations having irreversibly stretch-deformed diaphragms are also conceived and claimed as part of the scope of this invention.

FIG. 32 illustrates a duplex microfluidic cartridge 3060 formed of pneumatic and hydraulic circuits containing microvalves and micropumps of the invention. While the detailed operation of these circuits is beyond the scope of the description needed to appreciate the invention, one skilled in the art will understand that the inventive micropumps, microvalves, and microvents may be combined in larger assemblies of one, two or more microfluidic card devices and act cooperatively to function in biological assays, including extractions and purifications, and also amplification and detection steps, and are capable of performing multiple simultaneous

functions with very small fluid volumes. In this example, the larger of the two devices 3061 is an extraction subcircuit, and the smaller device 3602 is a detection subcircuit. Also shown is a gasket 3063 used in interfacing the microfluidic device pair through a common manifold to an off-card pneumatic actuation circuit under instrument control. To meet the challenge of higher throughput and assay complexity, increased miniaturization of these combinations is needed. The inventive diaphragms and subcombinations thereof result in improved miniaturization and increased circuit density, as is a desirable advance in the art.

FIG. 33 tabulates yield point for several common films useful in the microvalve diaphragms of the invention. Shown are yield points of exemplary stretchable diaphragm film materials, including linear low density polyethylenes, low density polyethylenes, ethylene vinyl acetate copolymers, polyvinylidene chloride, and SARANEX®. High density polyethylenes have a marginally higher yield strength but are readily modified to be suitable by a process of blending, grafting or co-lamination by skills known in the art. Suitable films for the yield-in-place process have yield strengths in the range of 2 to 30MPa, but films having higher yield points may be used with the mechanical processes described herein.

For example, also listed in the table for comparison is biaxially oriented polyethylene terephthalate, which has a narrow range of elasticity (elastic modulus of 1.35GPa) and a yield stress of about 100MPa. Films having a yield stress greater than 30MPa are not generally practical for yield-in-place processes at pressures and temperatures suitable for manufacturing of micropumps and microvalves unless modified due to practical constraints on applied pressure and temperature tolerated by the plastic cartridge body. Such films may be yielded using mechanical presses or rollers as described earlier, or equivalent processes known in the art.

Polycarbonates have relatively high yield strengths (in the range of 55 – 65MPa) and elastic modulus (about 2.3GPa). These materials are expected to resist elongation and are not generally suitable for yield-in-place operations within a cartridge body, but may be blended or laminated with more compliant materials, and may be stretched mechanically under suitable conditions.

Polyimides are generally stiff materials with an elastic modulus exceeding 4GPa and a yield strength of more than 70MPa. While unstretched polyimides have been used as diaphragms in microfluidic cartridges, their inherent stiffness is not compatible with reliable self-priming features and implies higher operating pressure than are generally practicable unless blended or otherwise

mechanically or pneumatically stretched prior to assembly to attain a suitable degree of flexibility.

Polyether ether ketone (PEEK) is generally not suitable. In addition to a yield stress of above 100MPa, the Young's modulus is greater than 3.6GPa, indicating an extremely stiff material that will not readily stretch without substantial applied force.

PTFE has no memory and is not an elastomer. However, the yield strength is relatively low (slightly more than 2MPa). Surprisingly, microporous forms of PTFE sold under the trade name MUPOR® are readily yielded in place using pneumatic pressure. These breathable films retain a significant plasticity after yield point is exceeded, and can be stretched to conform to a chamber using pressures in a range suitable for manufacturing of microassay cartridges. Microporous PTFE diaphragms that have been yielded in place may be operated as pumps or valves in the devices of the invention when wetted.

A large range of polyolefinic and related plastics have been found to be useful in forming stretch wraps and have yield strengths, toughness, and bonding characteristics suitable for use in the inventive microvalves and micropumps. Of particular interest are acrylates, vinyl chlorides, biaxially oriented polypropylene, and esters, for example. Polyvinylchloride may be used in blends and co-laminates. Use of polyolefins as blends and co-laminates to form "stretch wrap" films having the preferred yield strengths and bonding characteristics is well known in the art.

While the above is a description of the preferred embodiments of the present invention, it is possible to use various alternatives, modifications, combinations, and equivalents. Therefore, the scope of the present invention should be determined not with reference to the above description but should, instead, be determined with reference to the appended claims, along with their full scope of equivalents. The appended claims are not to be interpreted as including means-plus-function limitations, unless such a limitation is explicitly recited in a given claim using the phrase "means for."

All of the U.S. patents, U.S. patent application publications, U.S. patent applications, foreign patents, foreign patent applications and non-patent literature and publications referred to in this specification and/or cited in accompanying submissions, including but not limited to U.S. Patent Application No. 61/745,340, are incorporated herein by reference, in their entirety. Aspects of the embodiments may be modified, if necessary to employ concepts of the various patents, applications and publications to provide yet further embodiments. These and other changes may be made to the embodiments in light of the above-detailed description. In general, in the following

claims, the terms used should not be construed to limit the claims to the specific embodiments disclosed in the specification and the claims, but should be construed to include all possible embodiments along with the full scope of equivalents to which such claims are entitled. Accordingly, the claims are not limited by the specifics of the disclosure.

CLAIMS

What is claimed is:

1. A microfluidic cavity comprising a movable film separating two subcavities of the microfluidic cavity, said film having low elasticity, wherein the surface area of the film is larger than the cross-sectional area of the microcavity.

2. The microfluidic cavity film of claim 1, wherein the surface area of the film conforms in size to the inner surface area of one subcavity of said microcavity.

3. The microfluidic cavity of any one of claims 1-2, wherein said film is selected from:

a) a colaminate comprising a polyethylene terephthalate/ vinylidene chloride sandwich disposed between layers of polyethylene;

b) a polyethylene, a polyvinylidene chloride, a polyolefin, an ethylene vinyl acetate, and co-laminates and composites thereof; or,

c) a high density polyethylene, polyethylene terephthalate, a polycarbonate, a polyimide, a polyether ether ketone, a polypropylene, a polyvinylchloride, a polyacrylate, a polyester, and co-laminates and composites thereof.

4. The microfluidic cavity of any one of claims 1-3, wherein said film is a stretch wrap film, a co-laminate, or a composite thereof.

5. The microfluidic cavity of any one of claims 1-3, wherein said film is a microporous polymer.

6. The microfluidic cavity of any one of claims 1-5, wherein said two subcavities of said microfluidic cavity define a valve, said valve comprising

i) a first subcavity configured with a valve inlet, a valve outlet, and a valve seat interposed between said valve inlet and said valve outlet, wherein said first subcavity is configured to receive a fluid; and,

ii) a second subcavity configured to be reversibly pressurized.

7. The microfluidic cavity of claim 6, wherein said valve is fluidly joined to a microfluidic circuit.

8. The microfluidic cavity of any one of claims 1-5, wherein said two subcavities of said microfluidic cavity define a pump, said pump comprising

i) a first subcavity configured to receive a fluid; and,

ii) a second subcavity configured to be reversibly pressurized.

9. The microfluidic cavity of claim 8, wherein said pump is fluidly joined to a microfluidic circuit.

10. A method of making a film for separating microfluidic cavities in a microfluidic cartridge, the method comprising creating a low elasticity film by stretching a film beyond its yield point.

11. The method of claim 10, wherein the film is stretched in place within the microfluidic cartridge.

12. The method of any one of claims 10-11, wherein the film is stretched in the microfluidic cartridge during manufacture.

13. The method of any one of claims 10-11, wherein the film is stretched in the microfluidic cartridge after manufacture.

14. The method of claim 13, wherein the film is stretched in the microfluidic cartridge during initial operation.

15. The method of any one of claims 10-14, wherein the film is stretched by the application of a sufficiently large pressure difference to yield the film.

16. The method of any one of claims 10-15, wherein the microfluidic cartridge is fully assembled.

17. The method of any one of claims 10-15, wherein the microfluidic cartridge is partially assembled.

18. The method of claim 16, wherein the film is stretched by a pressure difference.

19. The method of claim 17, wherein the film is stretched by a mechanical force.

20. The method of claim 10, wherein the film is stretched by a die and then assembled into the microfluidic assembly.

21. The method of claim 10, wherein the film is stretched and then gathered into a shape complimentary to the microfluidic cavity using an external die.

22. The method of claim 10, wherein the film is stretched and then gathered into a shape complimentary to the microfluidic cavity using the microfluidic cavity.

23. A micropump, said micropump comprising a cavity having

i) a first subcavity configured to receive a fluid;

ii) a second subcavity configured to be reversibly pressurized;

iii) a diaphragm interposed between and separating said first subcavity from said second subcavity; and,

wherein said diaphragm is a polymeric thin film web having a yield point and is characterized by a permanently overstretched deformation of said web.

24. The micropump of claim 23, wherein said thin film web is a low density polyethylene / ethylene vinyl acetate/polyvinylidene chloride/ ethylene vinyl acetate / low density polyethylene co-laminate film.

25. The micropump of claim 23, wherein said thin film is composed of polyethylene, polyvinylidene chloride, poly-ethylene vinyl acetate, polyvinylchloride, polypropylene, polyolefin, or a composite or a co-laminate thereof.

26. The micropump of claim 23, wherein said thin film web is a high density polyethylene, polyethylene terephthalate, a polycarbonate, a polyimide, a polyether ether ketone, a polypropylene, a polyvinylchloride, a polyacrylate, a polyester, or a co-laminate or a composite thereof.

27. The micropump of claim 23, wherein said thin film web is a stretch-wrap film.

28. The micropump of claim 23, wherein said thin film web is a chemically resistant polymer.

29. The micropump of any one of claims 23-28, wherein said micropump is self-priming.

30. The micropump of any one of claims 23-29, wherein said micropump is manufactured by a process of locally overstretching said web under a force applied to said thin film, said force being in excess of said yield point.

31. The micropump of any one of claims 23-30, wherein said thin film web inelastically conforms in a first state to a first internal surface of said cavity when pressurized and in a second state to a second internal surface of said cavity when depressurized.

32. The micropump of claim 31, wherein said micropump is configured to pump a liquid according to a pump stroke defined by the reversible motion of said permanently overstretched deformation of said web between said first state and said second state as driven by pressurization and depressurization of said second subcavity.

33. The micropump of claim 32, wherein said first subcavity comprises a port configured for receiving a liquid.

34. The micropump of claim 32, wherein said first subcavity comprises a port for discharging a liquid.

35. The micropump of any one of claims 23-34, wherein the micropump is enclosed in a disposable cartridge body of a microassay device.

36. The micropump of claim 30, wherein said force is a pneumatic force applied to said diaphragm after assembly of the disposable cartridge body.

37. The micropump of claim 30, wherein said web is first overstretched by locally applying a force; and then said cavity is assembled by aligning said web with said cavity while apposingly mating said first subcavity with said second subcavity.

38. The micropump of claim 30, wherein said web is overstretched by a process of forcing said web into a subcavity; and then said cavity is assembled by apposingly mating said first subcavity with said second subcavity.

39. The micropump of claim 30, wherein said force is a mechanical force applied under controlled process conditions.

40. A microvalve, said microvalve comprising a cavity having

i) a first subcavity configured with a valve inlet, a valve outlet, and a valve seat interposed between said valve inlet and said valve outlet, wherein said first subcavity is configured to receive a fluid;

ii) a second subcavity configured to be reversibly pressurized;

iii) a diaphragm interposed between and separating said first subcavity from said second subcavity, wherein said diaphragm is enabled to be reversibly deflected against said valve seat according to whether said second subcavity is pressurized or depressurized, thereby defining an "ON" position and an "OFF" position of said microvalve; and,

further characterized in that said diaphragm is a polymeric thin film web having a yield point and is characterized by a permanently overstretched deformation of said web.

41. The microvalve of claim 40, wherein said thin film web is a low density polyethylene/ ethylene vinyl acetate/polyvinylidene chloride/ ethylene vinyl acetate /low density polyethylene co-laminate film.

42. The microvalve of claim 40, wherein said thin film is composed of polyethylene, polyvinylidene chloride, poly-ethylene vinyl acetate, polyvinylchloride, polypropylene, polyolefin, or a composite or a co-laminate thereof.

43. The microvalve of claim 40, wherein said thin film web is a high density polyethylene, polyethylene terephthalate, a polycarbonate, a polyimide, a polyether ether ketone, a polypropylene, a polyvinylchloride, a polyacrylate, a polyester, or a co-laminate or a composite thereof.

44. The microvalve of claim 40, wherein said thin film web is a stretch-wrap film.

45. The microvalve of claim 40, wherein said thin film web is a chemically resistant polymer.

46. The microvalve of any one of claims 40-45, wherein said microvalve is manufactured by a process of locally overstretching said web under a force applied to said thin film, said force being in excess of said yield point.

47. The microvalve of claim 46, wherein said force is a pressure applied under controlled process conditions.

48. The microvalve of claim 46, wherein said force is a mechanical force applied under controlled process conditions.

49. The microvalve of any one of claims 40-48, wherein said thin film web inelastically conforms in a first state to a first internal surface of said cavity when pressurized and in a second state to a second internal surface of said cavity when depressurized.

50. The microvalve of claim 49, wherein said microvalve is configured to open and close said microvalve by the reversible motion of said permanently overstretched deformation of said web between said first state and said second state as driven by pressurization and depressurization of said second subcavity.

51. The microvalve of any one of claims 40-50, wherein said first subcavity comprises a port configured for receiving a liquid.

52. The microvalve of any one of claims 40-50, wherein said first subcavity comprises a port for discharging a liquid.

53. The microvalve of any one of claims 40-52, wherein the microvalve is enclosed in a disposable cartridge body of a microassay device.

54. The microvalve of claim 46, wherein said force is a pneumatic force applied to said diaphragm after assembly of the disposable cartridge body.

55. The microvalve of claim 46, wherein said web is first overstretched by locally applying a force; and then said cavity is assembled by aligning said web with said cavity while apposingly mating said first subcavity with said second subcavity.

56. The microvalve of claim 46, wherein said web is overstretched by a process of forcing said web into a subcavity; and then said cavity is assembled by apposingly mating said first subcavity with said second subcavity.

57. A microassay device having a disposable cartridge body enclosing a micropump in a cavity therein, said micropump comprising

i) a first subcavity configured to receive a liquid from a fluidic circuit;

ii) a second subcavity configured to receive a pneumatic pressure;

iii) a pneumohydraulic diaphragm interposed between and separating said first subcavity from said second subcavity; and,

wherein said diaphragm comprises a thin film web of a breathable microporous elastomer.

58. The microassay device of claim 57, wherein said cavity is formed at a terminus of a fluidic circuit fluidly joined to said first subcavity.

59. The microassay device of claims 57 or 58, wherein said microporous elastomer is a breathable hydrophobic polyurethane.

60. The microassay device of claims 57 or 58, wherein said microporous elastomer is a breathable hydrophilic polyurethane.

61. The microassay device of claims 57 or 58, wherein said pneumatic pressure is a positive pressure or a suction pressure.

62. A microassay device having a disposable cartridge body enclosing a self-venting micropump in a cavity therein, said micropump comprising

a) a hydraulic subcavity, wherein said hydraulic subcavity defines a first surface of said cavity;

b) a pneumatic subcavity, wherein said pneumatic subcavity defines a second surface of said cavity;

c) a diaphragm disposed in said cavity and separating said pneumatic subcavity from said hydraulic subcavity;

d) a fluidic channel entering said hydraulic subcavity through said first surface, wherein said fluidic channel is configured as the sole fluid inlet and outlet of said hydraulic subcavity;

e) a pneumatic channel or vent entering said pneumatic subcavity through said second surface; and,

wherein said diaphragm comprises a thin film web of a breathable polymer.

63. The device of claim 62, wherein said breathable polymer is a microporous polyurethane.

64. The device of claim 62, wherein said breathable polymer is a microporous polytetrafluoroether.

65. The device of any one of claims 62-64, wherein said pneumatic channel is a vent and said diaphragm is an elastomer operative as a passive pump operating by elastically storing energy when distended under pressure and elastically releasing energy when relaxed.

66. The device of any one of claims 62-65, wherein said diaphragm comprises a thin film web of a breathable polymer

67. The device of any one of claims 62-66, wherein said pneumatic channel is operatively connected to a pneumatic pulse generator enabled to apply a train of suction pressure pulses to said diaphragm.

68. The device of any one of claims 62-66, wherein said pneumatic channel is operatively connected to a pneumatic pulse generator enabled to apply alternating positive and suction pressure pulses to said diaphragm.

69. A device for exchanging a gas in a liquid, said device comprising

a) a body having a cavity therein, said cavity comprising

i) a hydraulic subcavity, wherein said hydraulic subcavity defines a first surface of said cavity;

ii) a pneumatic subcavity, wherein said pneumatic subcavity defines a second surface of said cavity;

iii) a thin film web disposed in said cavity and separating said pneumatic subcavity from said hydraulic subcavity;

b) a circuit enabled to flow the liquid from a first fluidic channel to a second fluidic channel through said hydraulic subcavity, wherein said fluidic channels are fluidly connected to said hydraulic subcavity through ports in said first surface;

c) a pneumatic channel entering said pneumatic subcavity through a port in said second surface, wherein said pneumatic channel is enabled to admit or evacuate the gas; and,

wherein said thin film web is a breathable polymer.

70. The device of claim 69, wherein said breathable polymer is a microporous elastomer.

71. The device of claim 69, wherein said breathable polymer is a microporous polyurethane.

72. The device of claim 69, wherein said breathable polymer is a microporous polytetrafluoroether.

73. The device of any one of claims 69-72, wherein said pneumatic channel is configured to enable evacuating the gas from the pneumatic subcavity at a reduced pressure.

74. The device of claim 73, wherein the device is configured for degassing the fluid flowing under the thin film web.

75. The device of any one of claims 69-72, wherein said pneumatic channel is configured to enable admitting the gas into the pneumatic subcavity at a positive pressure.

76. The device of claim 75, wherein said pneumatic channel is configured to inject a bubble into the flowing fluid.

Fig. 1A

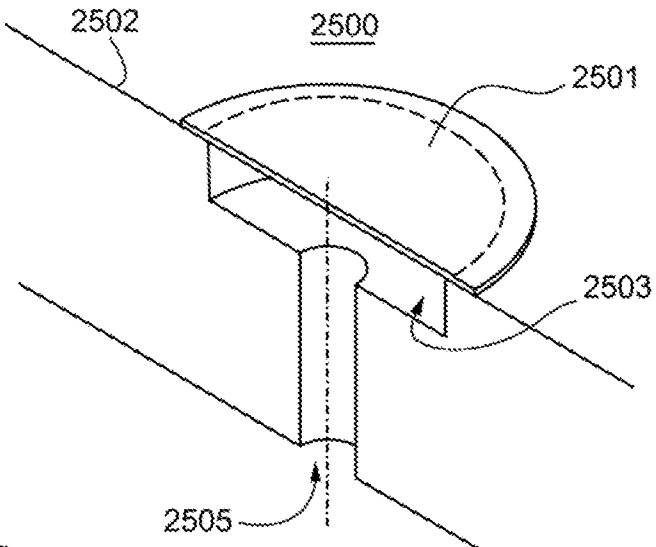


Fig. 1B

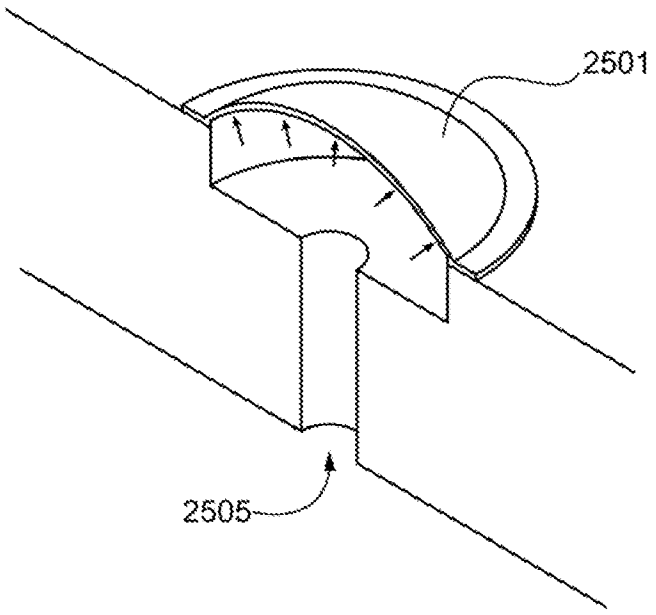


Fig. 2A

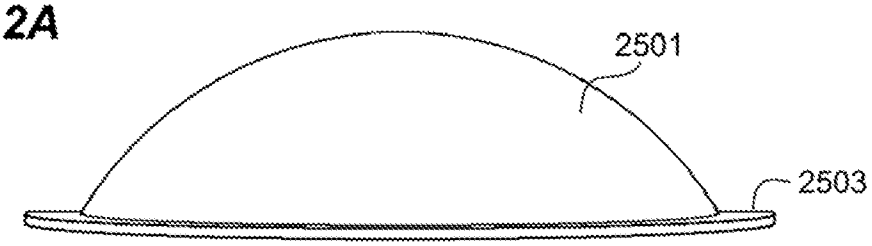


Fig. 2B

Fig. 3

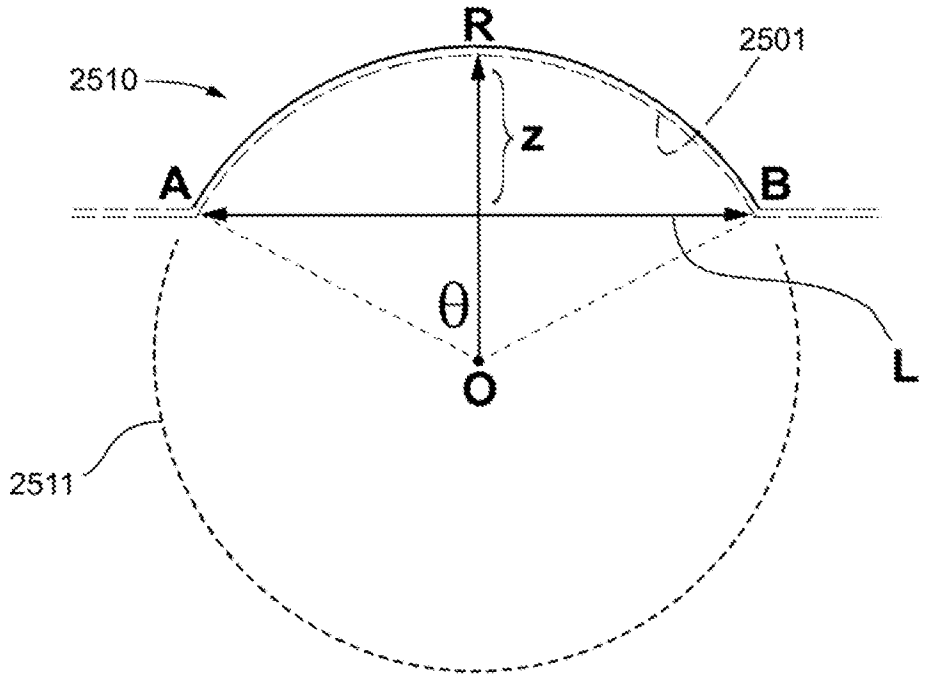


Fig. 4A

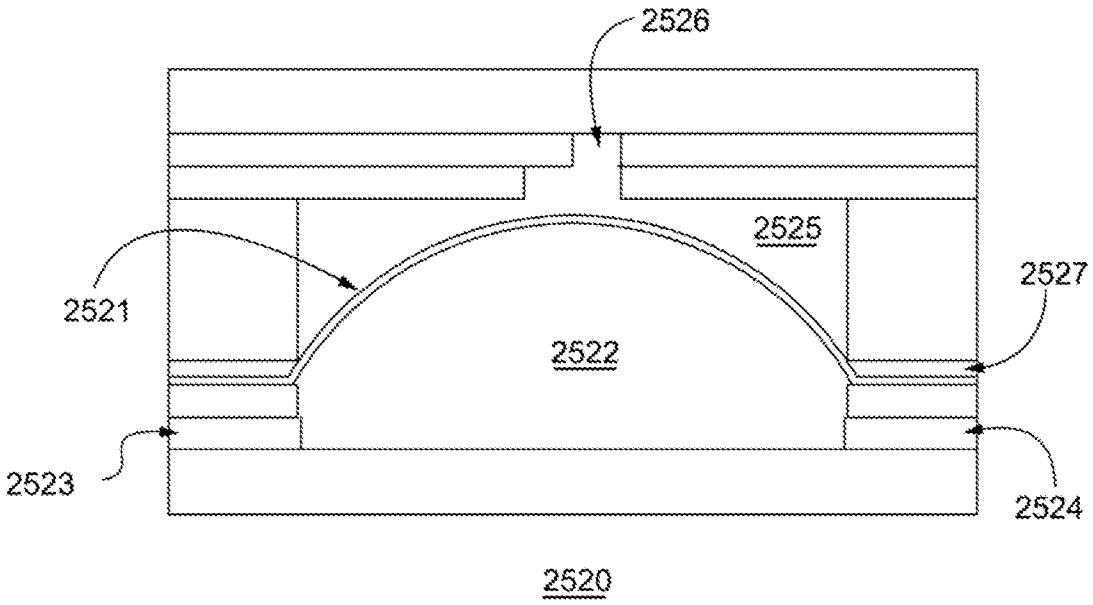


Fig. 4B

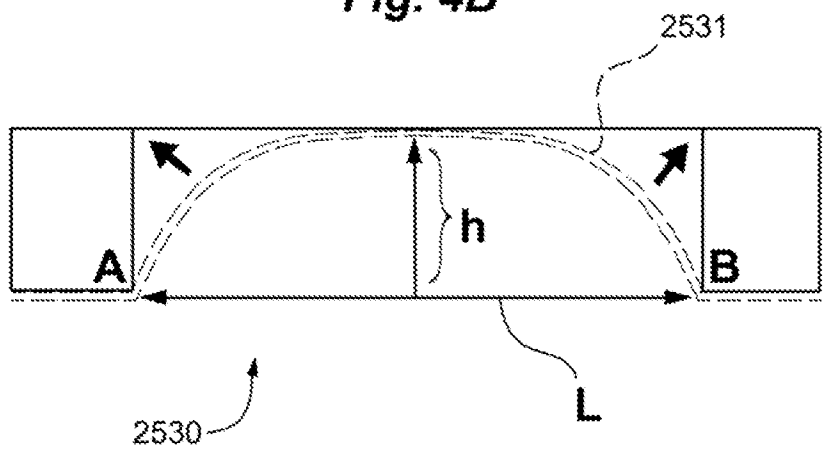


Fig. 5A

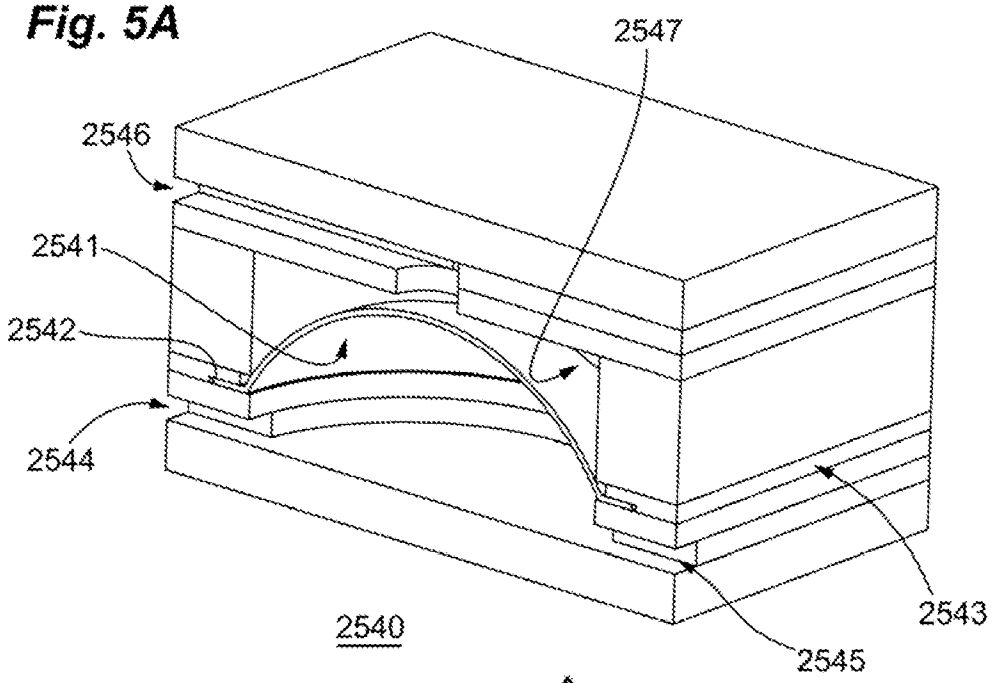

2547
2546
2541
2542
2544
2543
2545
2540

Fig. 5B

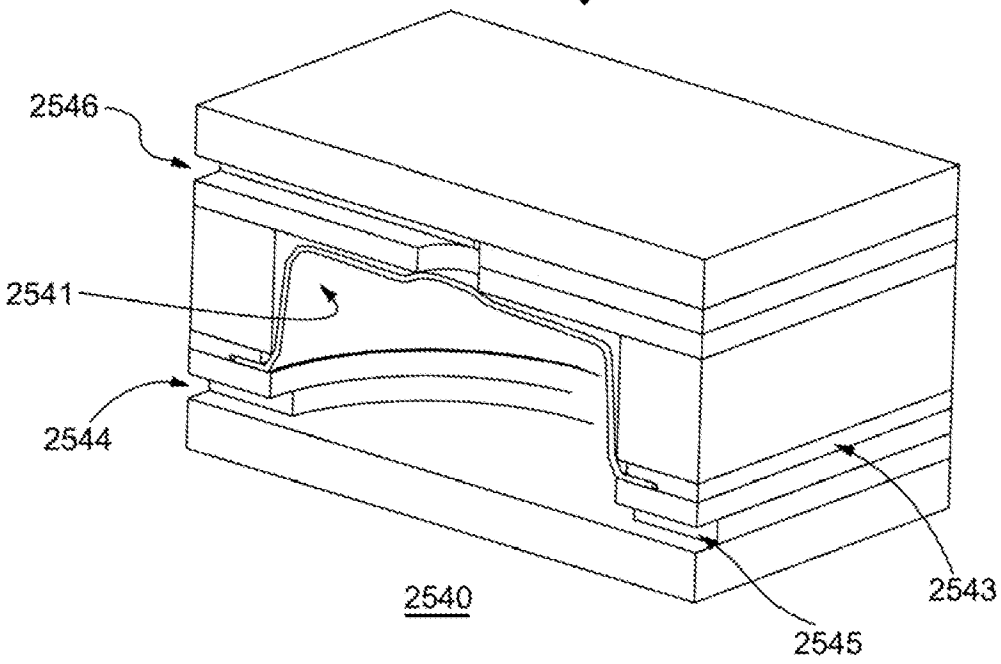

2546
2541
2544
2543
2545
2540

Fig. 6A

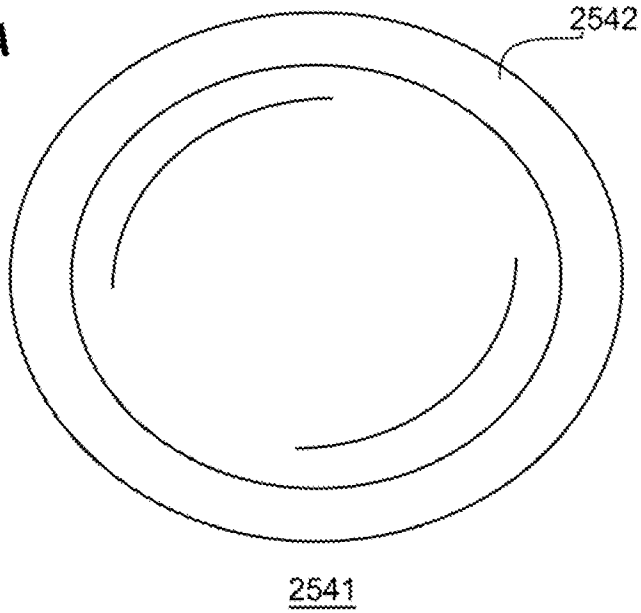


Fig. 6B

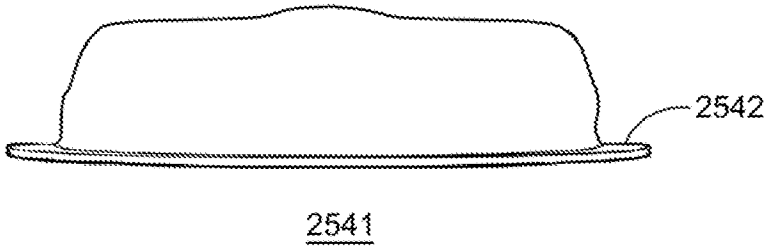


Fig. 6C

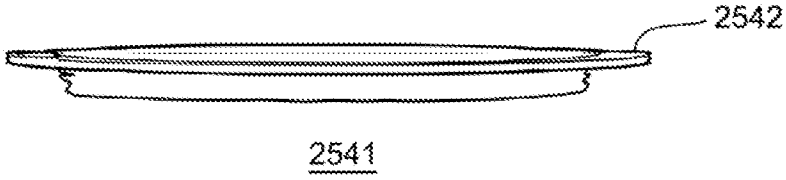


Fig. 7

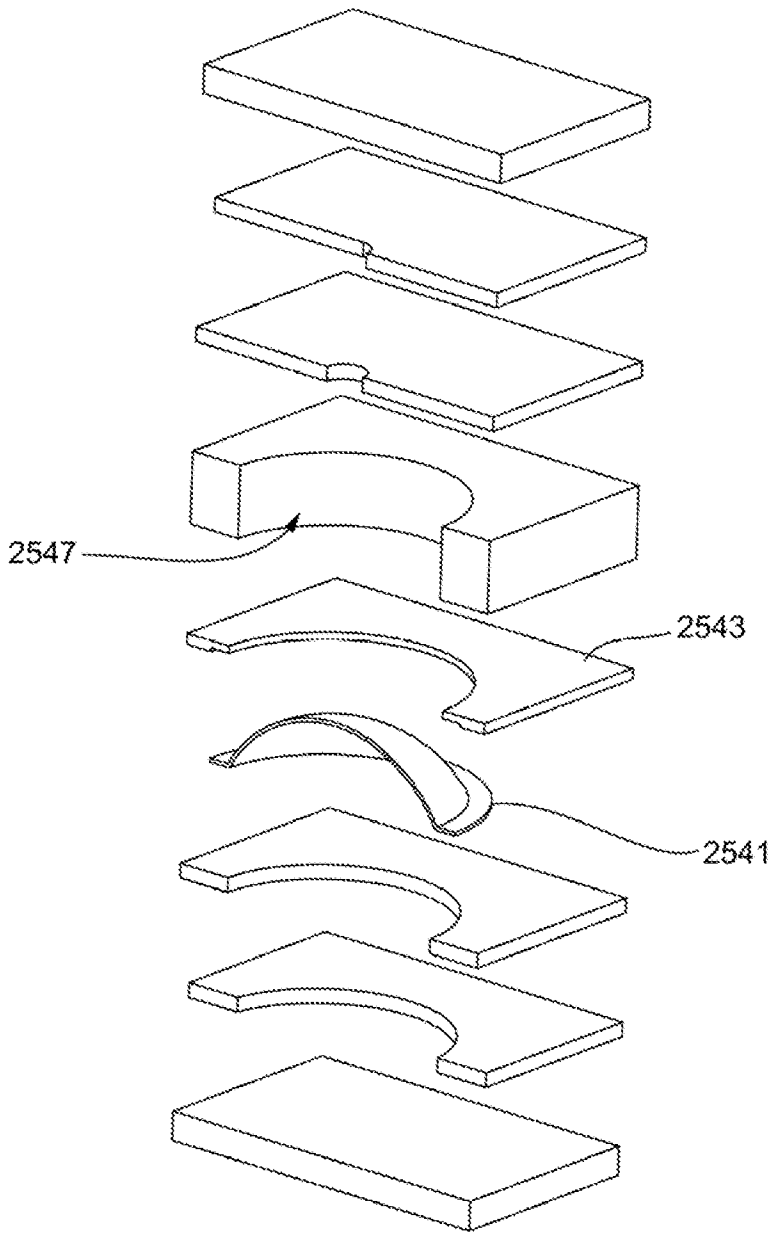
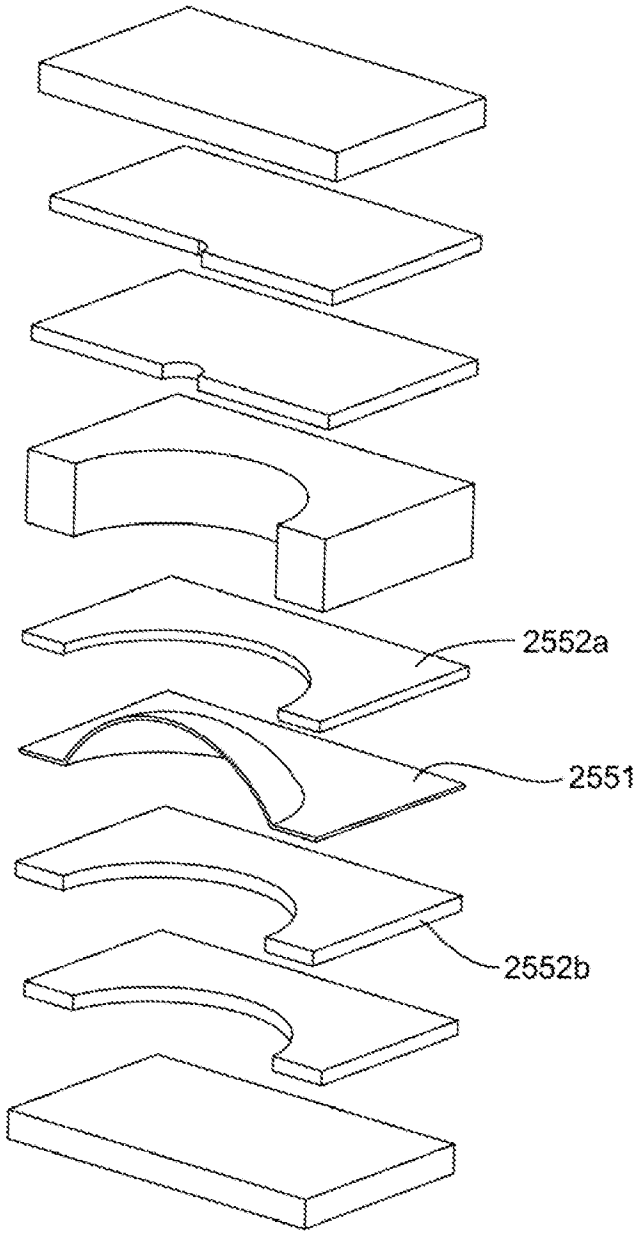


Fig. 8



2550

Fig. 9A

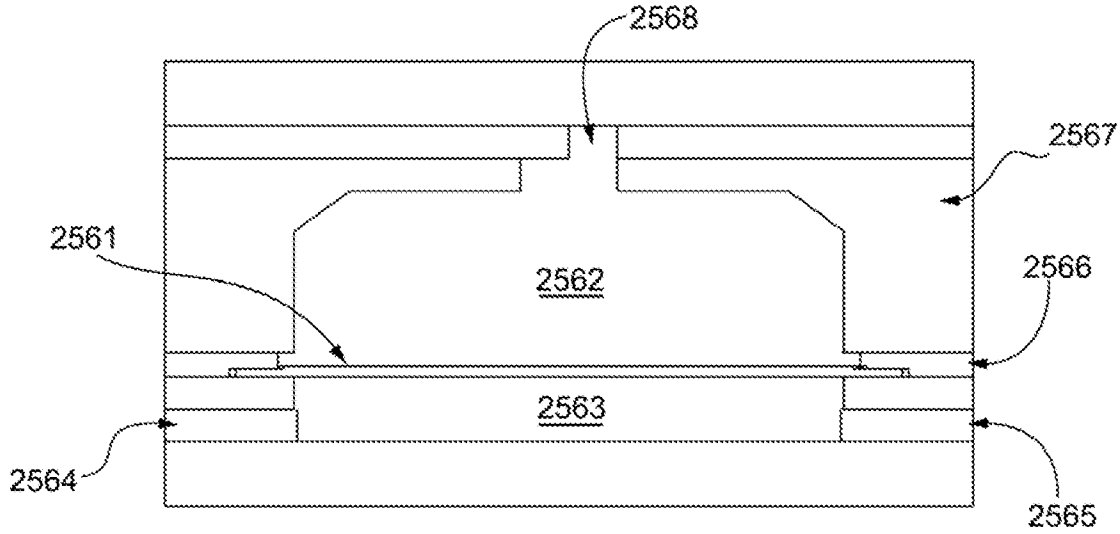

2568
2567
2561
2562
2566
2563
2564
2565

Fig. 9B

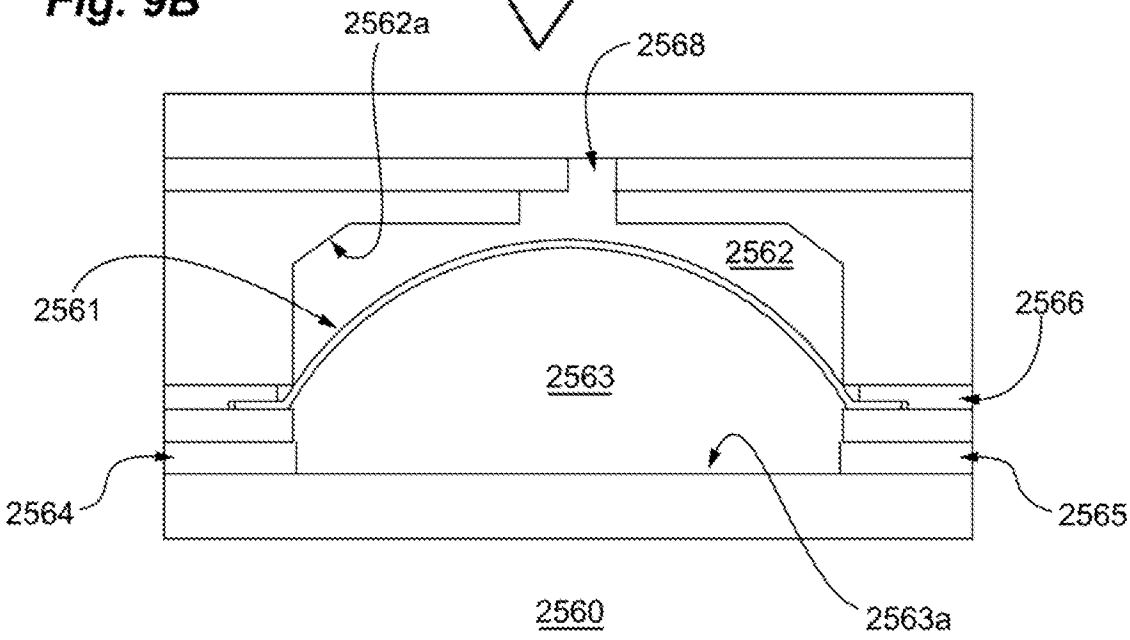

2562a
2568
2562
2561
2566
2563
2564
2565
2560
2563a

Fig. 10A

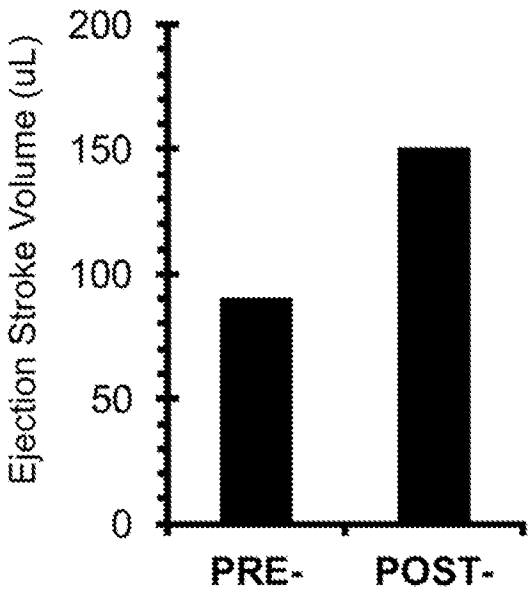


Fig. 10B

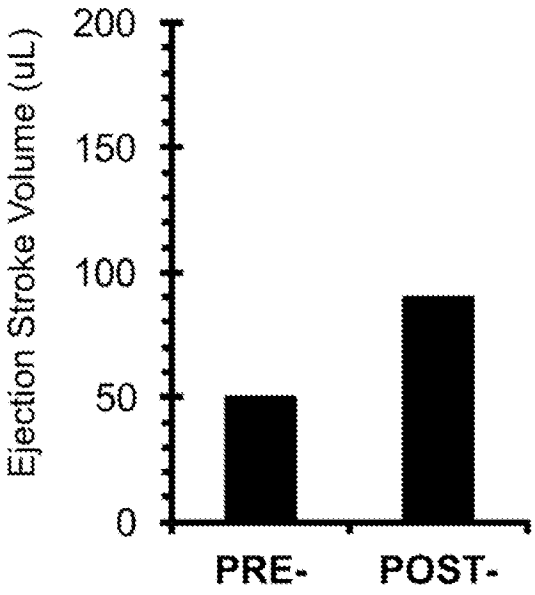


Fig. 11

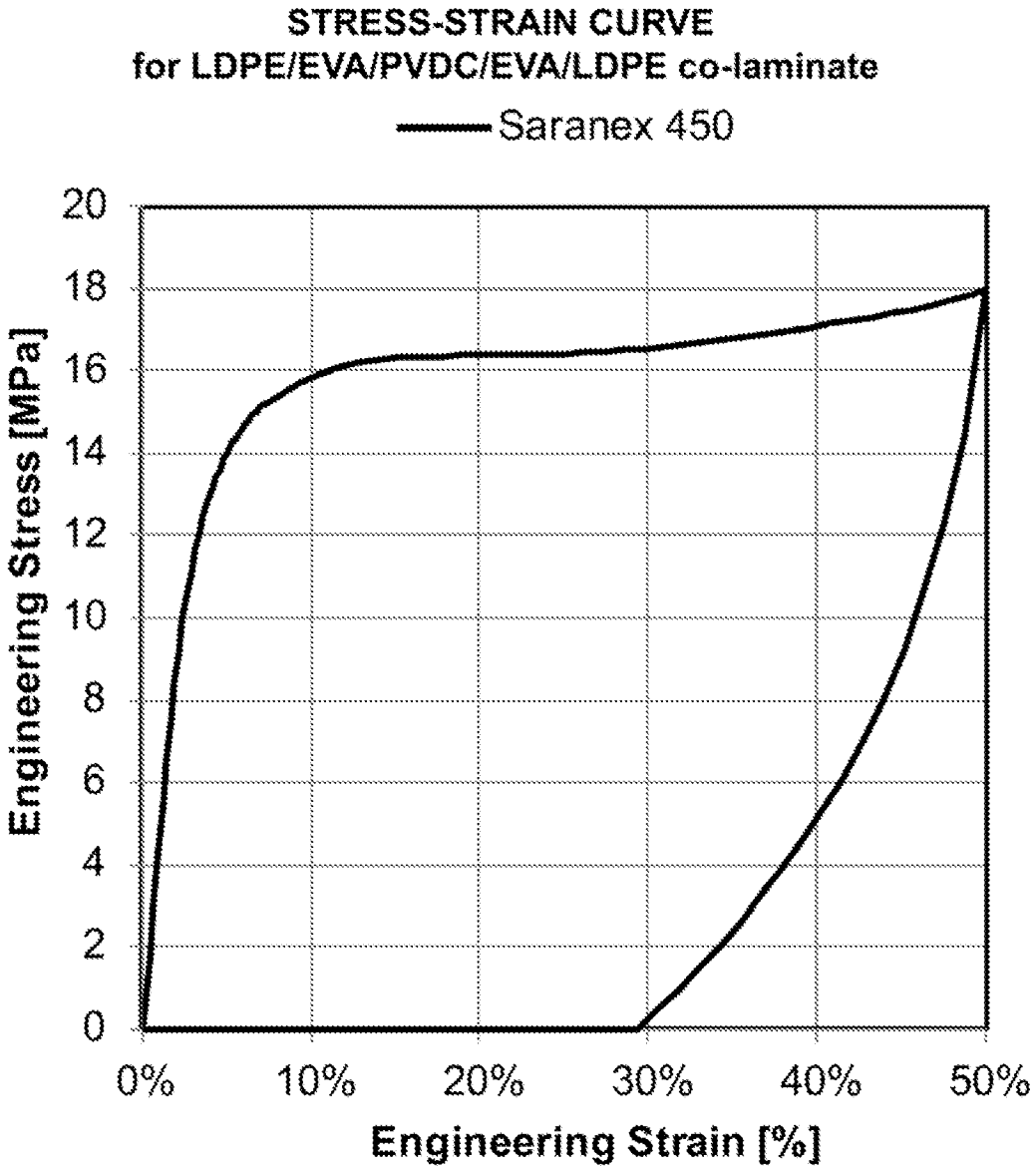

STRESS-STRAIN CURVE
for LDPE/EVA/PVDC/EVA/LDPE co-laminate
Saranex 450
Engineering Stress [MPa]
0
2
4
6
8
10
12
14
16
18
20
0%
10%
20%
30%
40%
50%
Engineering Strain [%]

Fig. 12

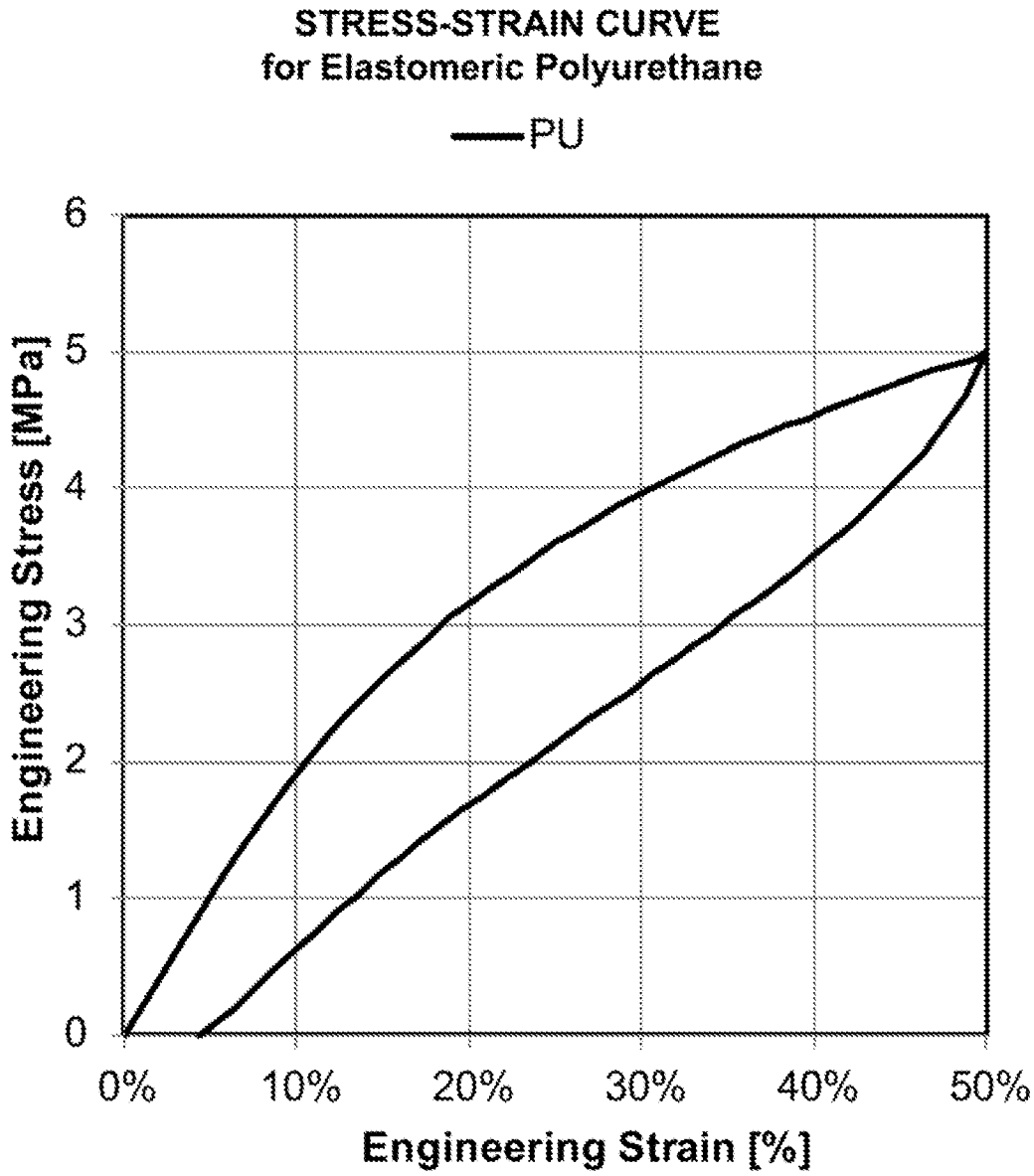

STRESS-STRAIN CURVE
for Elastomeric Polyurethane
PU
Engineering Stress [MPa]
0
1
2
3
4
5
6
0%
10%
20%
30%
40%
50%
Engineering Strain [%]

Fig. 13

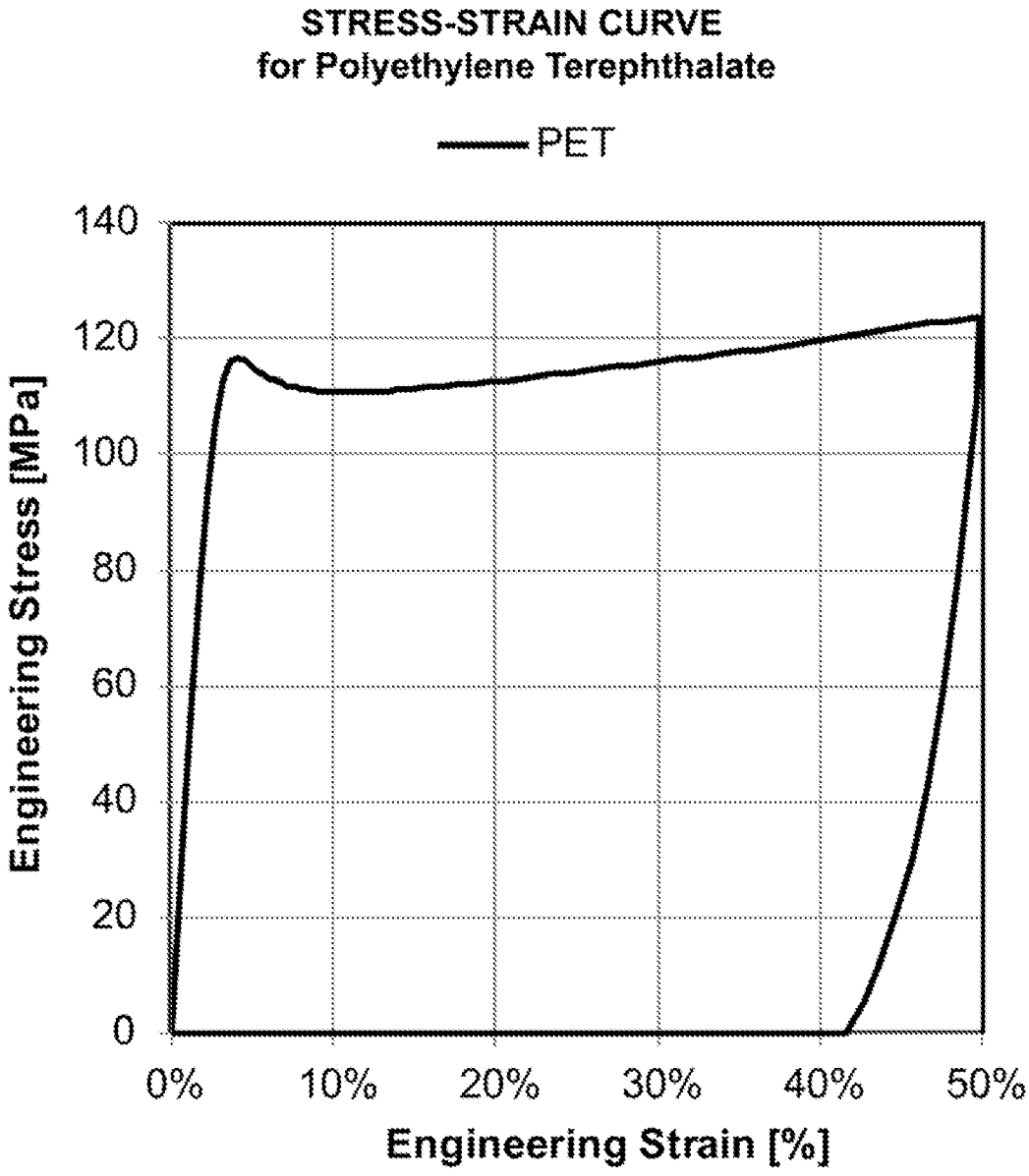

STRESS-STRAIN CURVE
for Polyethylene Terephthalate
PET
Engineering Stress [MPa]
140
120
100
80
60
40
20
0
0%
10%
20%
30%
40%
50%
Engineering Strain [%]

Fig. 14

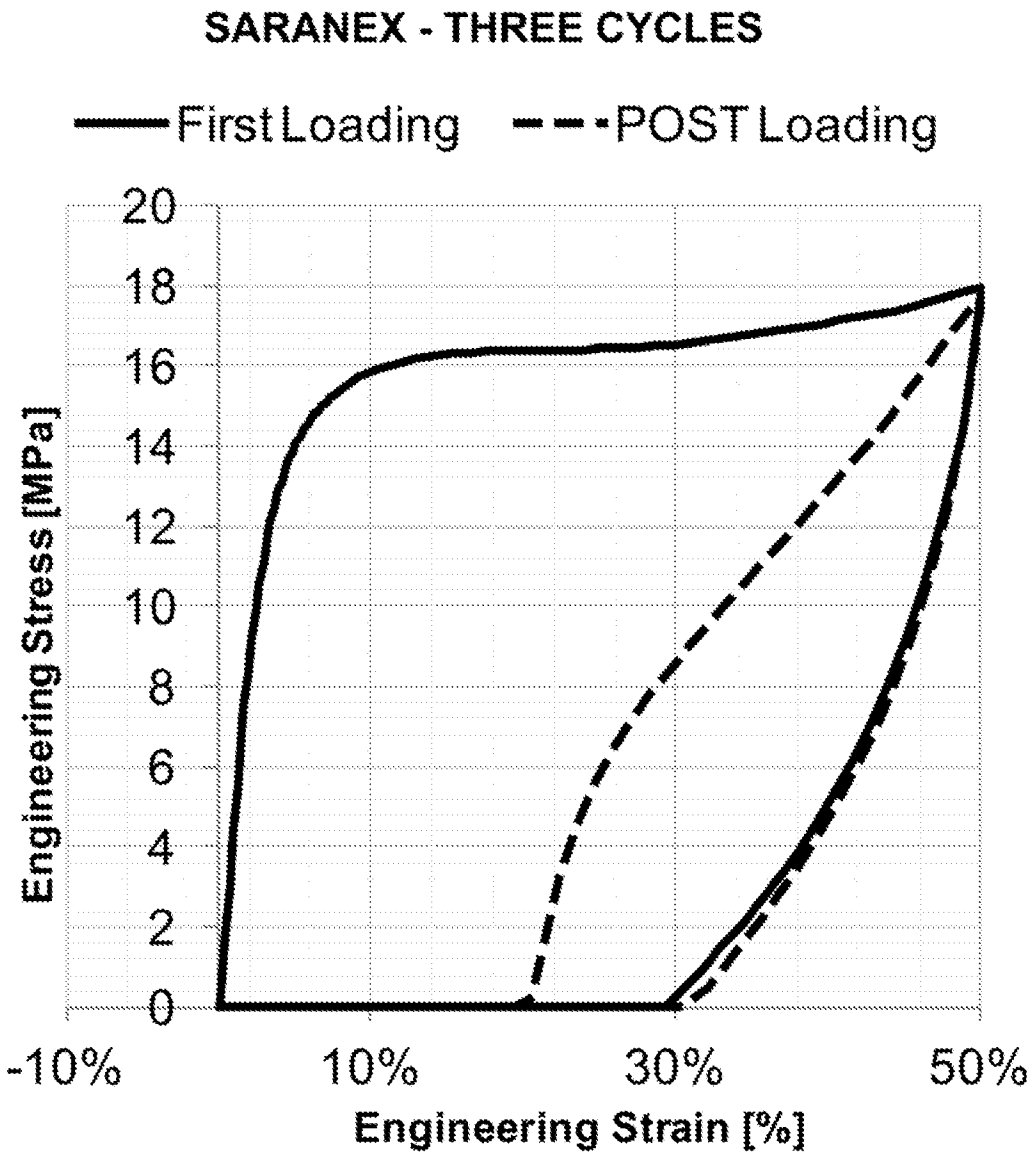

SARANEX - THREE CYCLES
First Loading
POST Loading
Engineering Stress [MPa]
20
18
16
14
12
10
8
6
4
2
0
-10%
10%
30%
50%
Engineering Strain [%]

Fig. 15

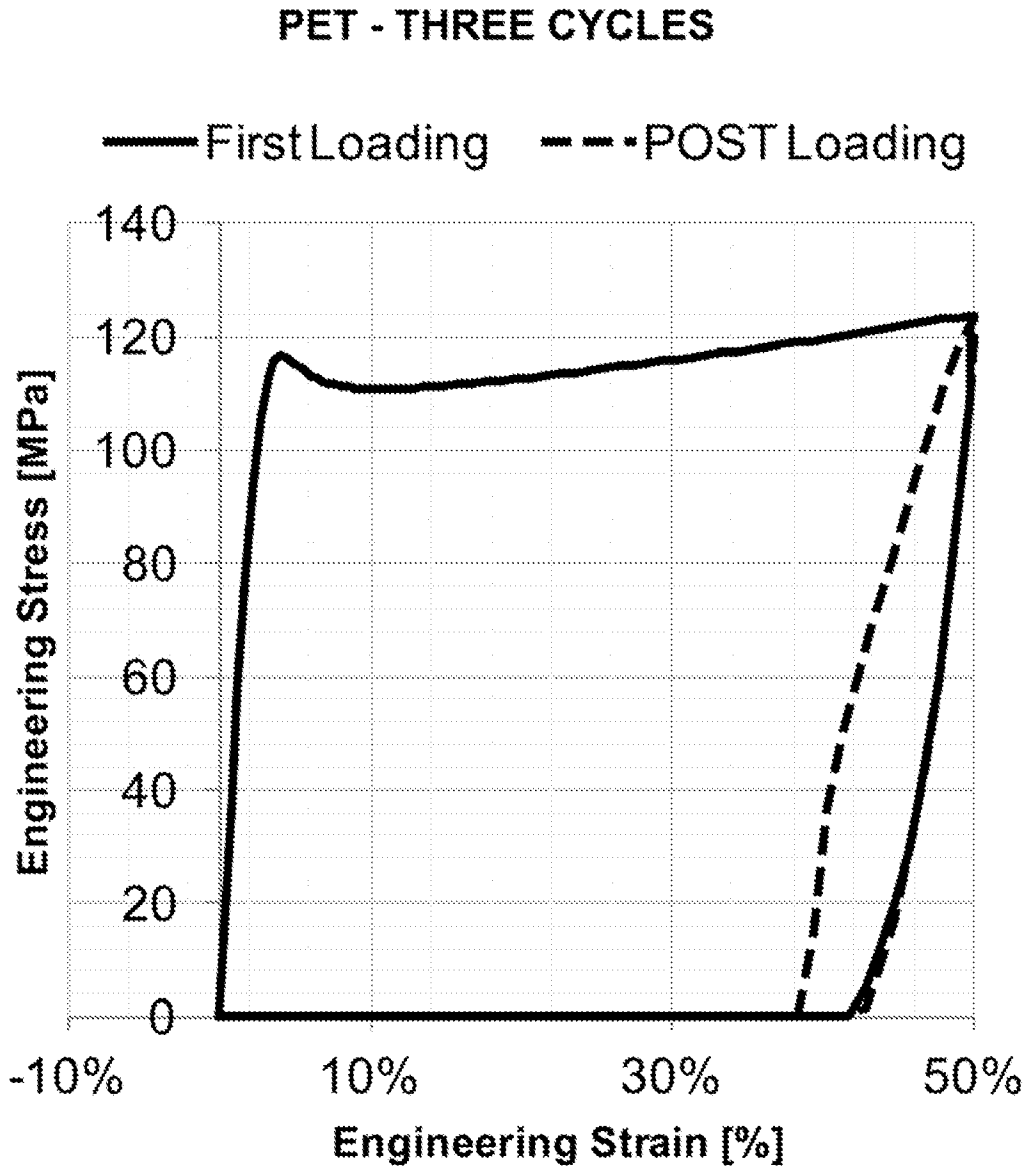
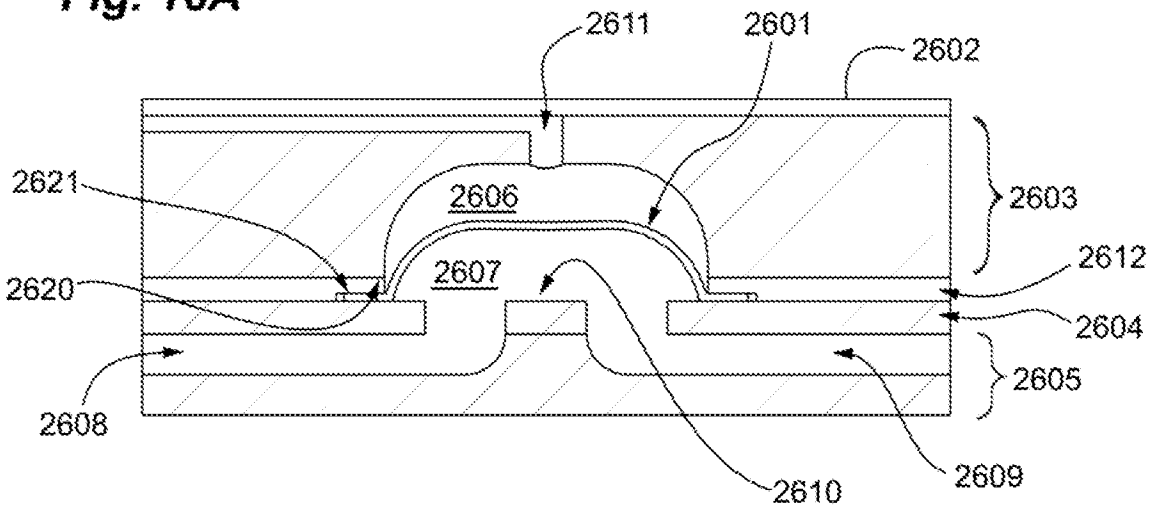

PET - THREE CYCLES
First Loading
POST Loading
140
120
100
80
60
40
20
0
Engineering Stress [MPa]
-10%
10%
30%
50%
Engineering Strain [%]

Fig. 16A



"OPEN"

"OFF"

Fig. 16B

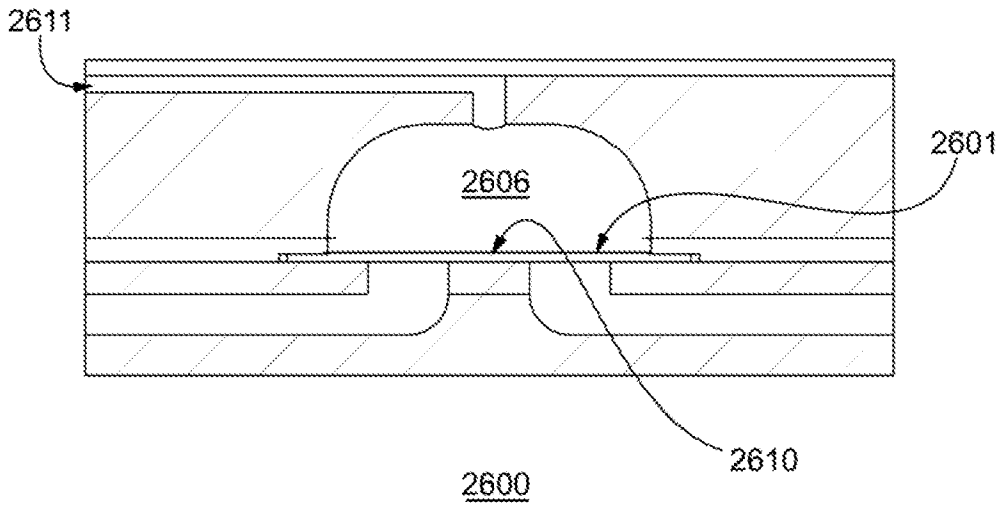


Fig. 17

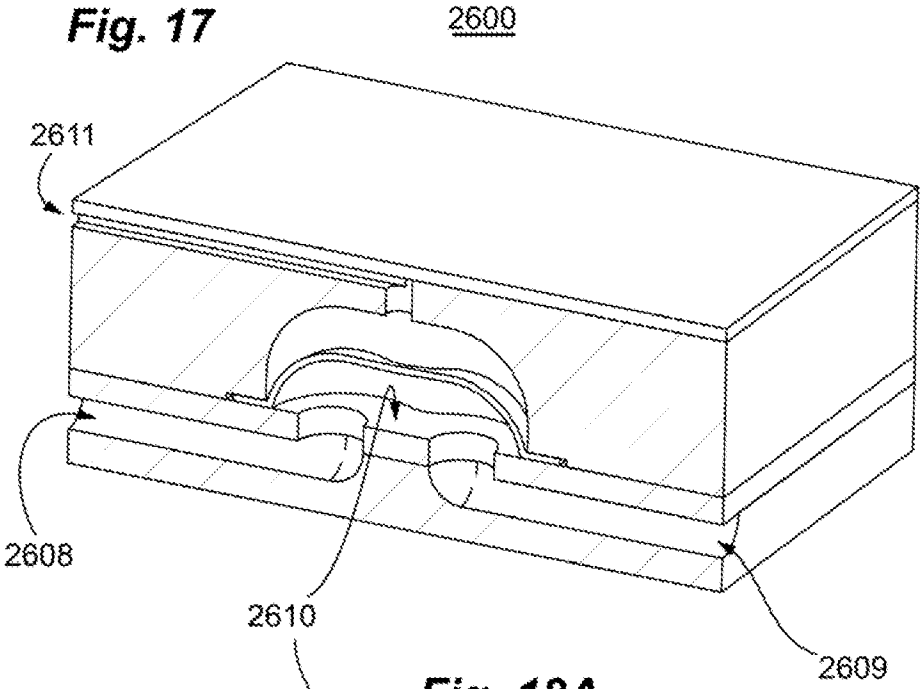


Fig. 18A

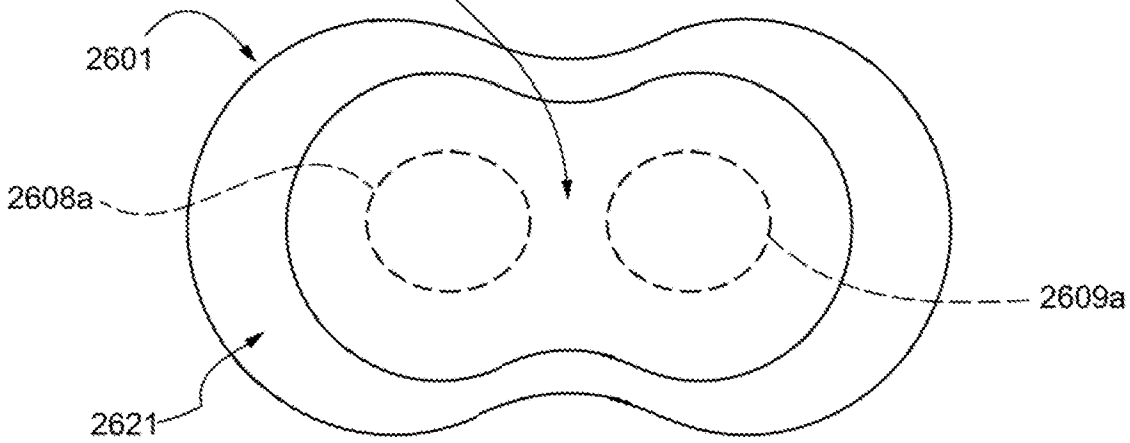


Fig. 18B

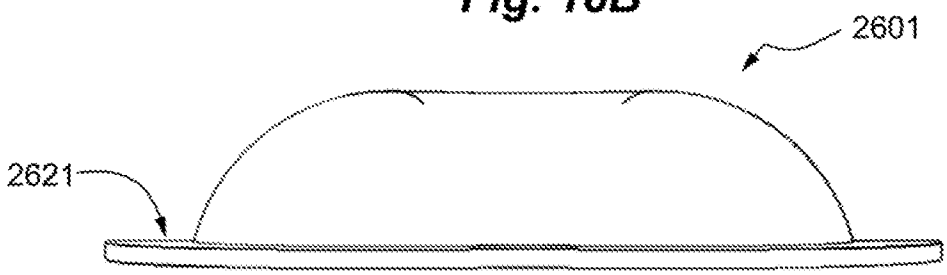


Fig. 19

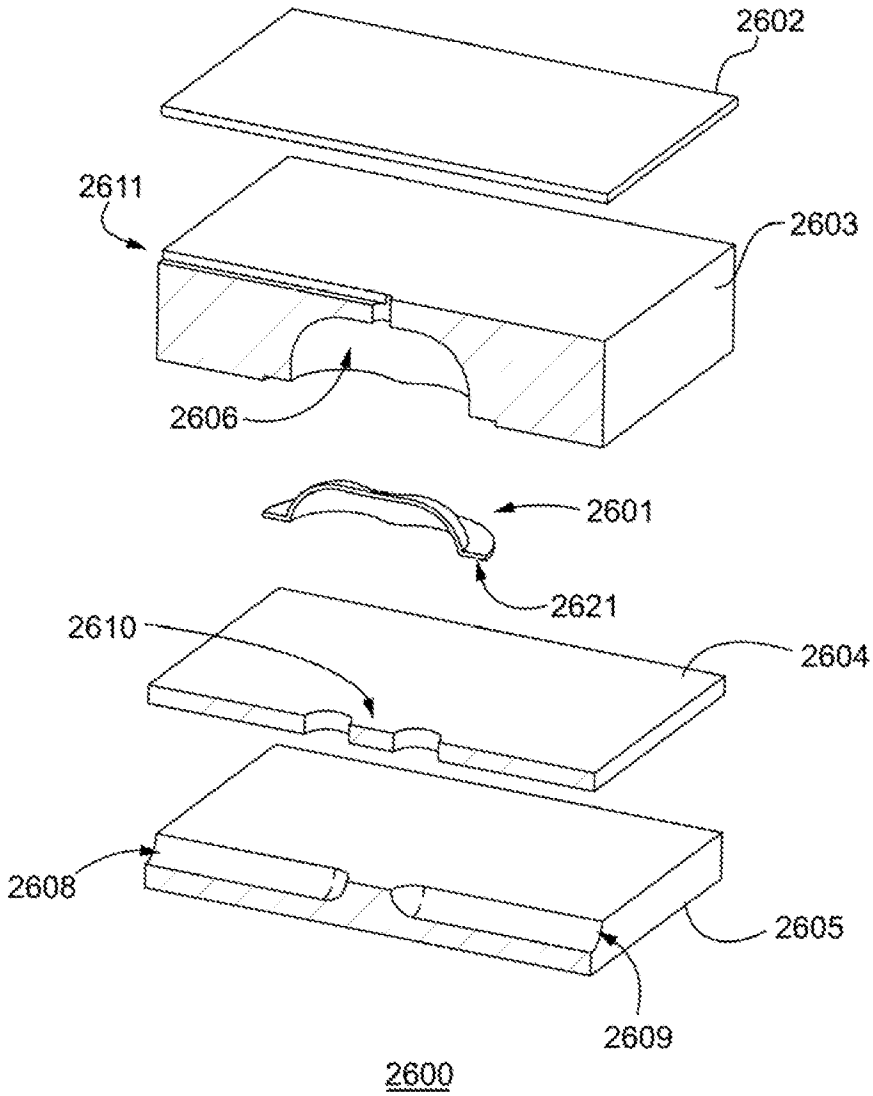


Fig. 20A

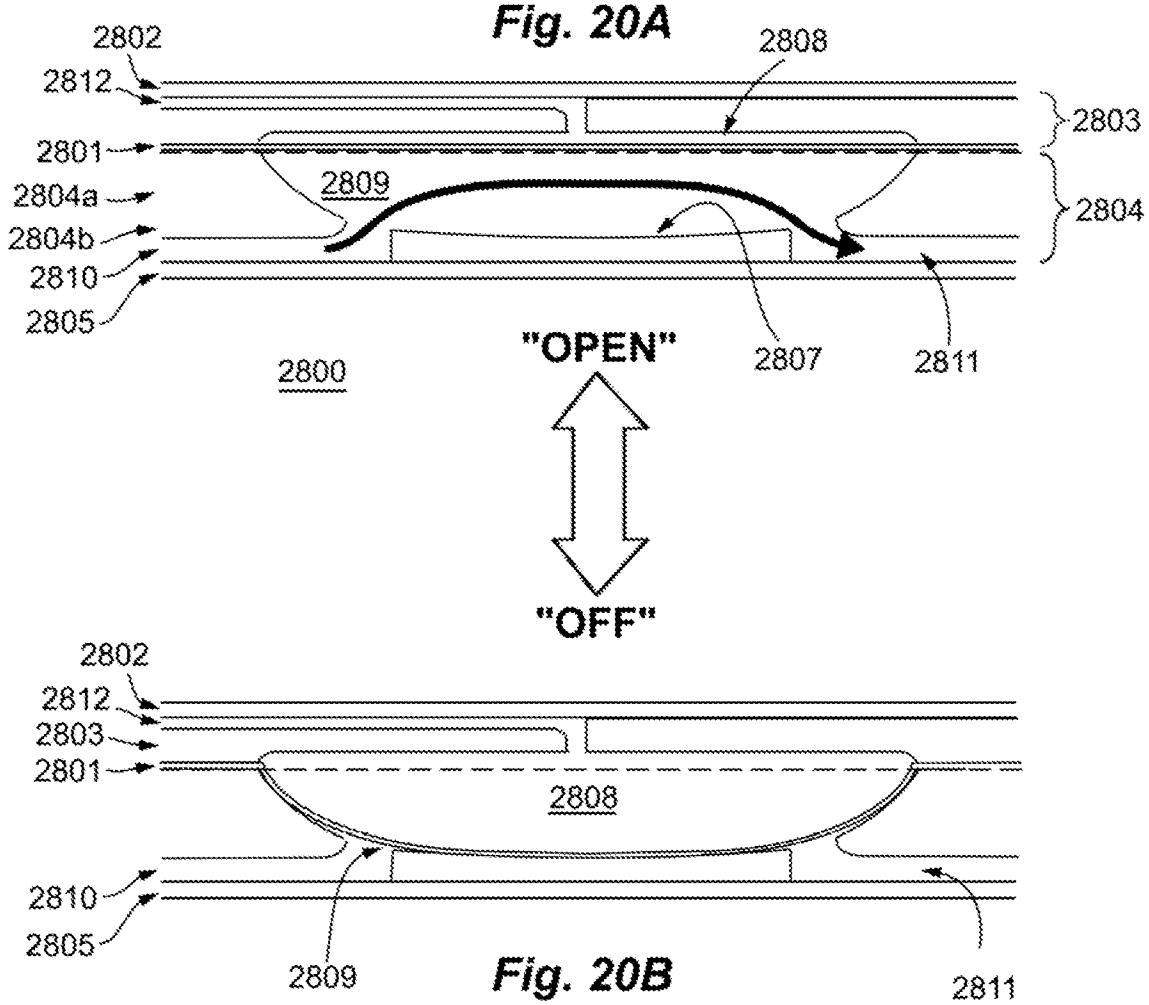


Fig. 20B

Fig. 21

Fig. 22A

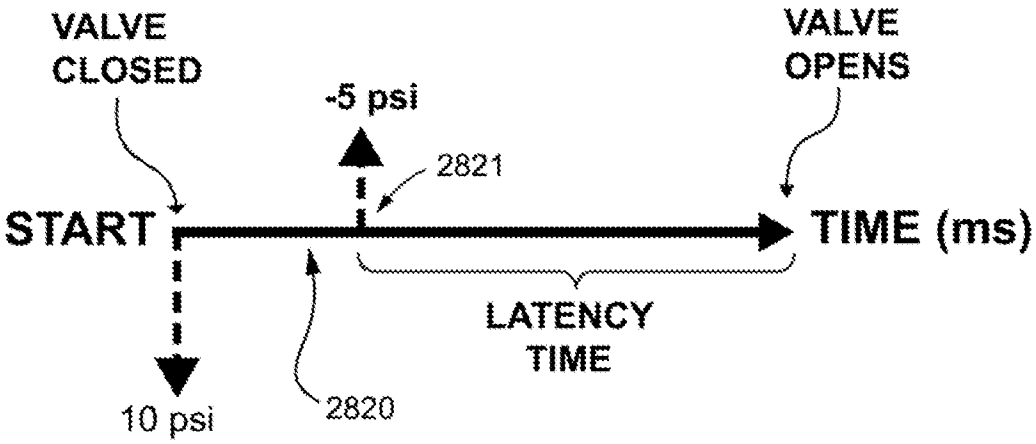


Fig. 22B

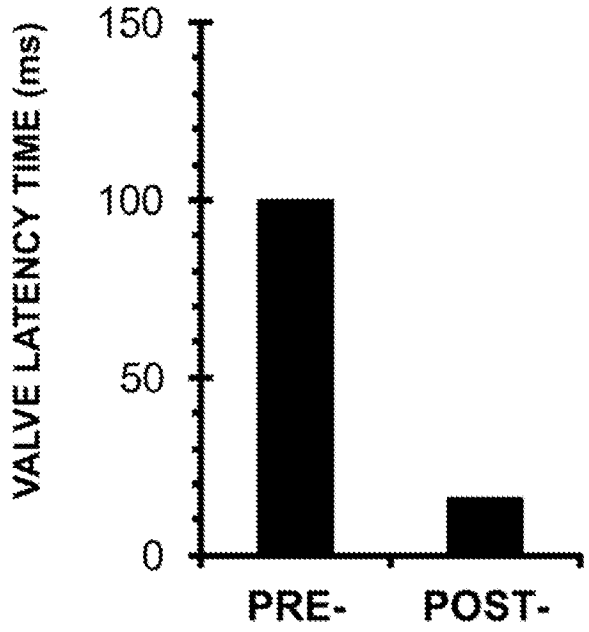


Fig. 23

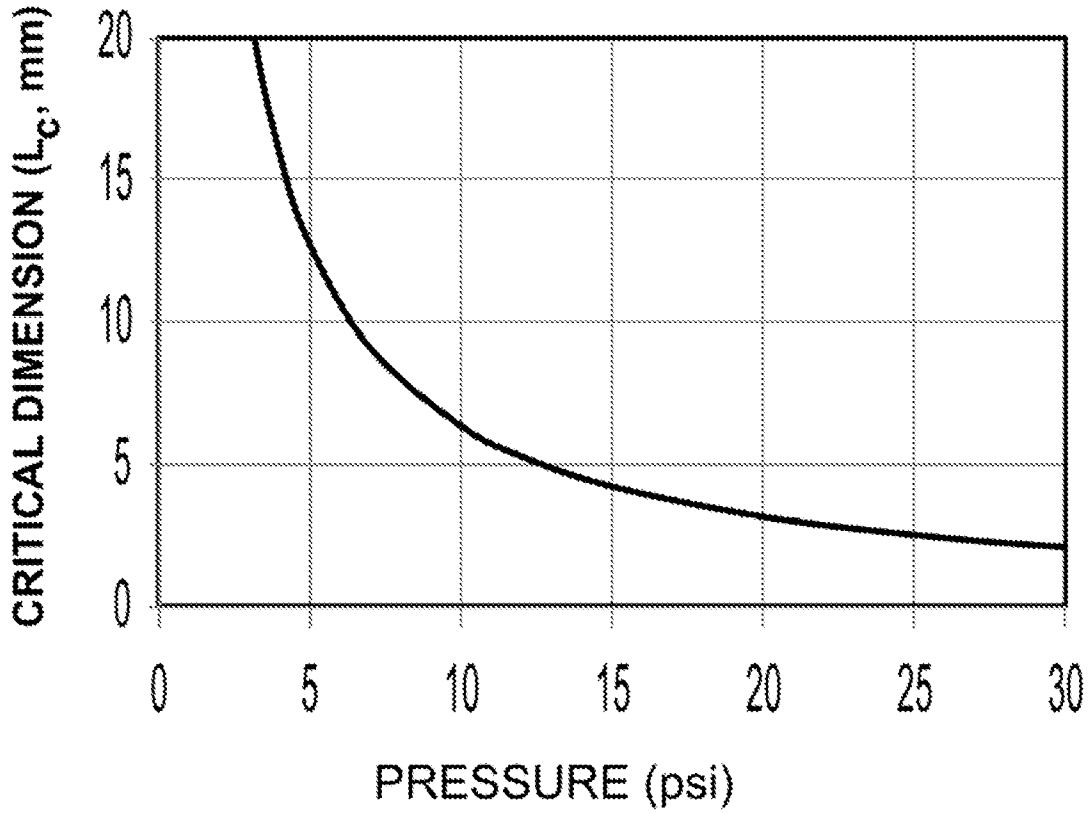

CRITICAL DIMENSION (L_c, mm)
20
15
10
5
0
0
5
10
15
20
25
30
PRESSURE (psi)

Fig. 24

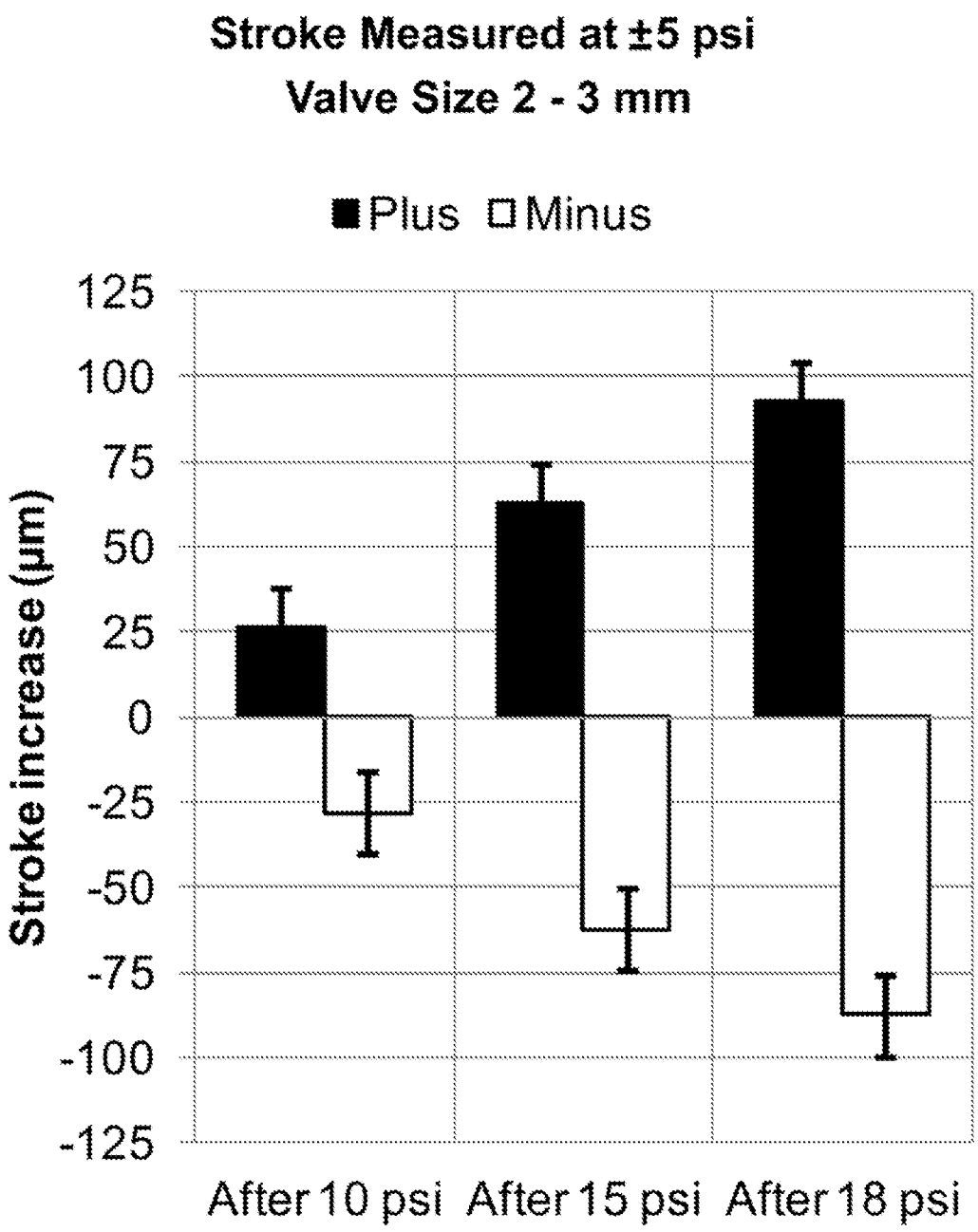

Stroke Measured at ±5 psi
Valve Size 2 - 3 mm
Plus
Minus
Stroke increase (µm)
125
100
75
50
25
0
-25
-50
-75
-100
-125
After 10 psi
After 15 psi
After 18 psi

Fig. 25A

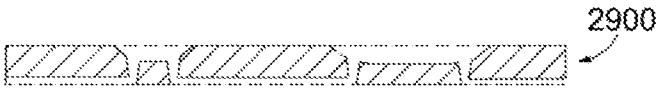


Fig. 25B

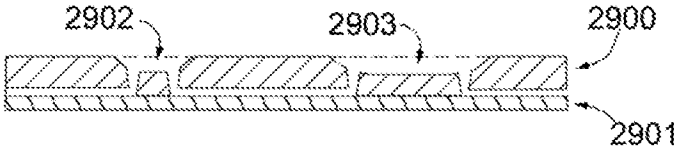


Fig. 25C

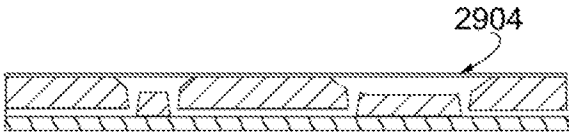


Fig. 25D

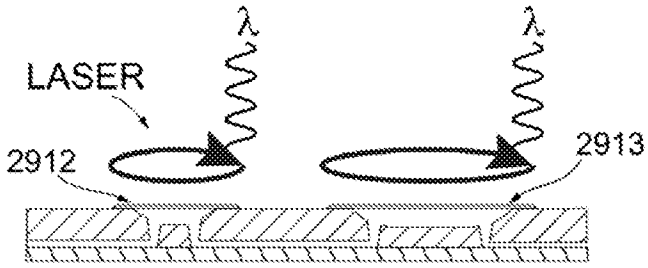


Fig. 25E

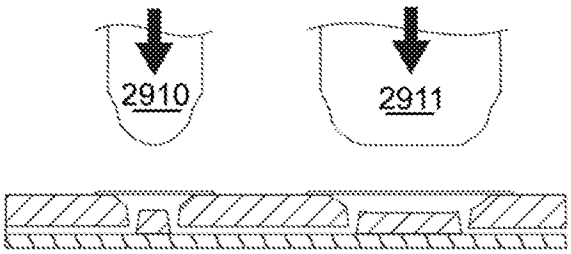


Fig. 25F

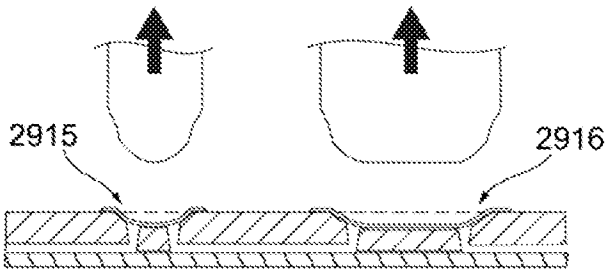


Fig. 26A

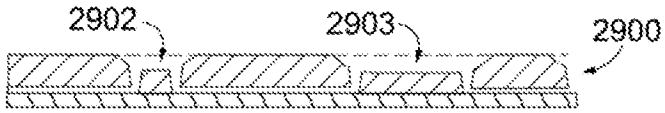


Fig. 26B

Fig. 26C

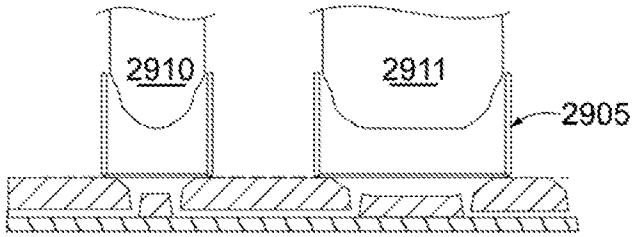


Fig. 26D

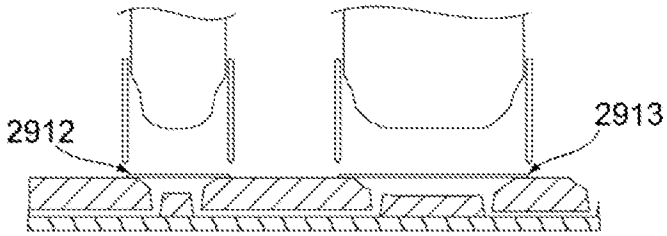


Fig. 26E

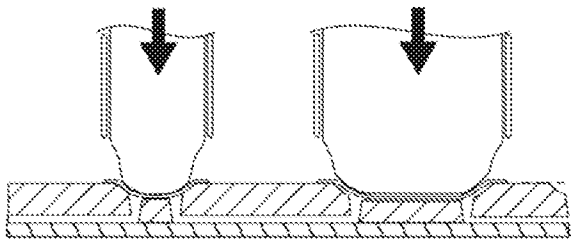


Fig. 26F

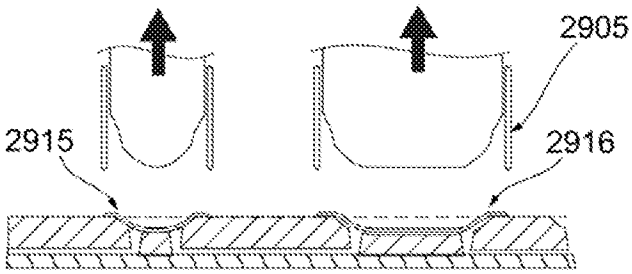


Fig. 27A

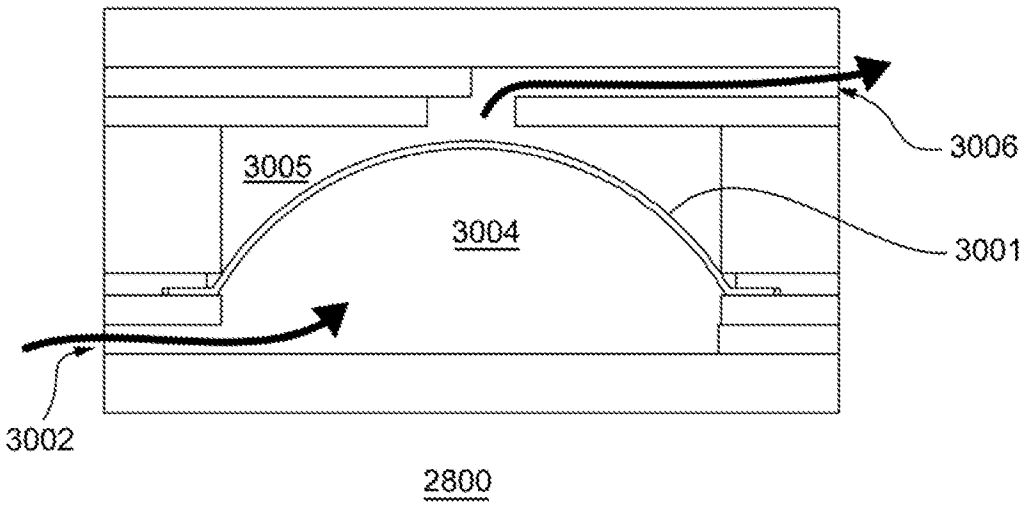


Fig. 27B

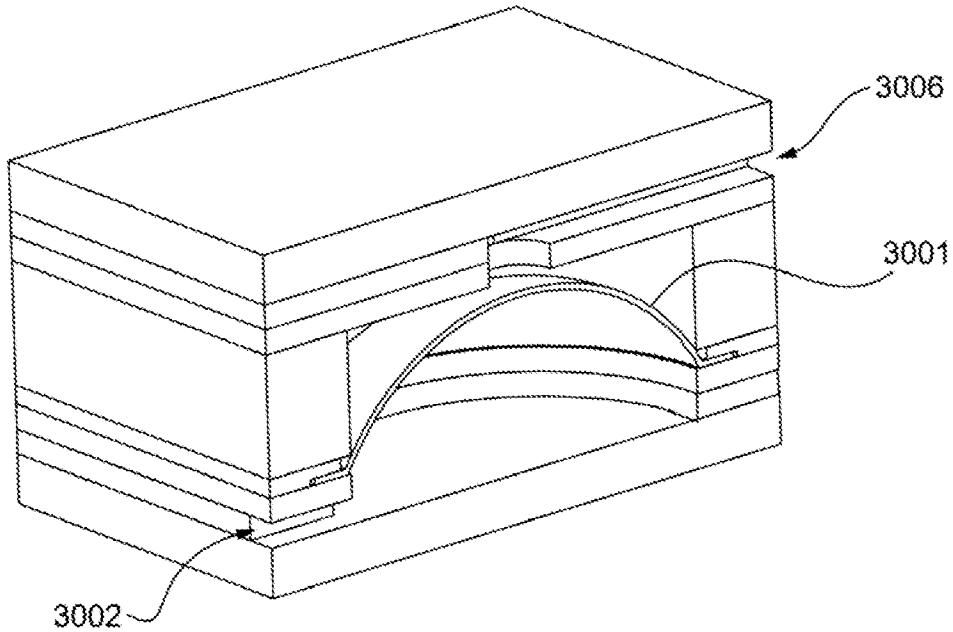


Fig. 28

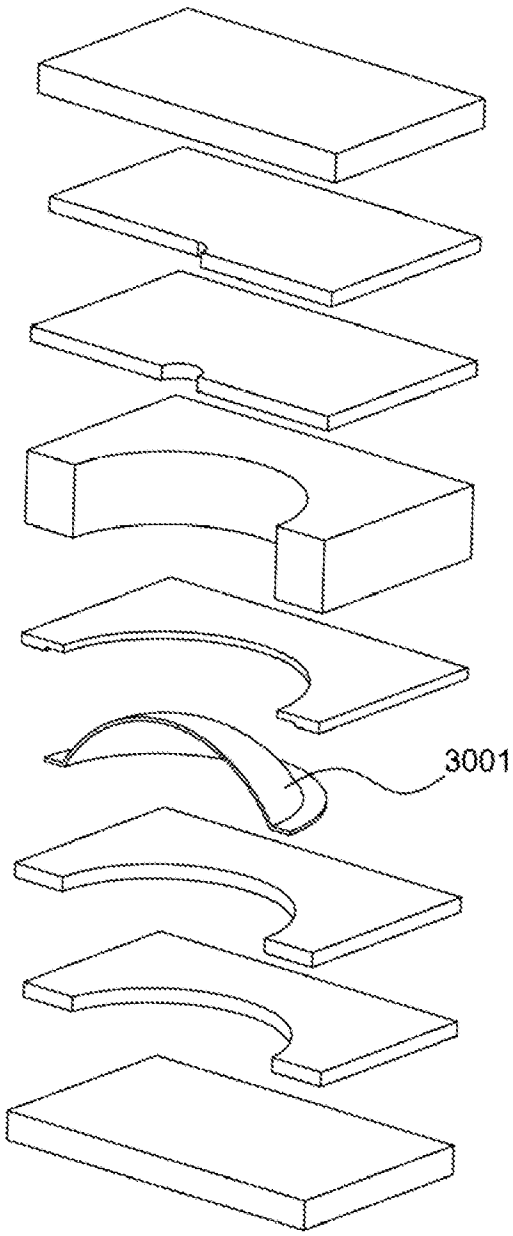
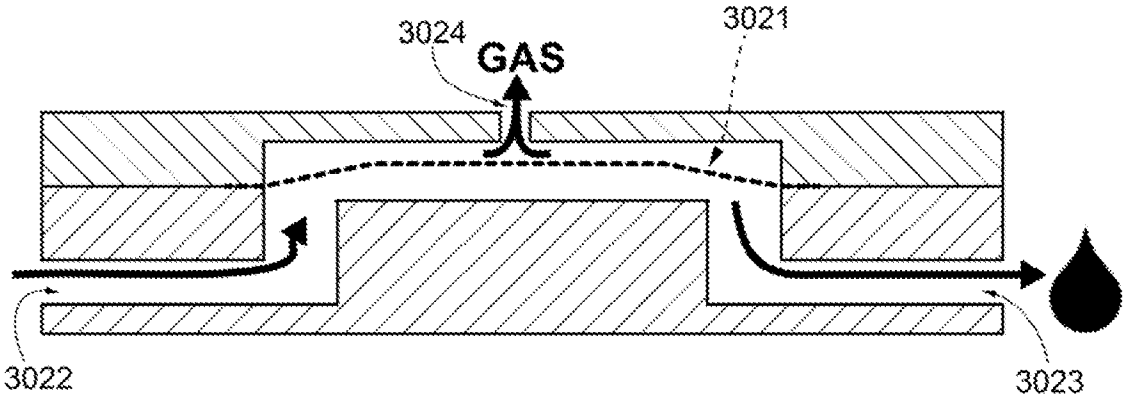
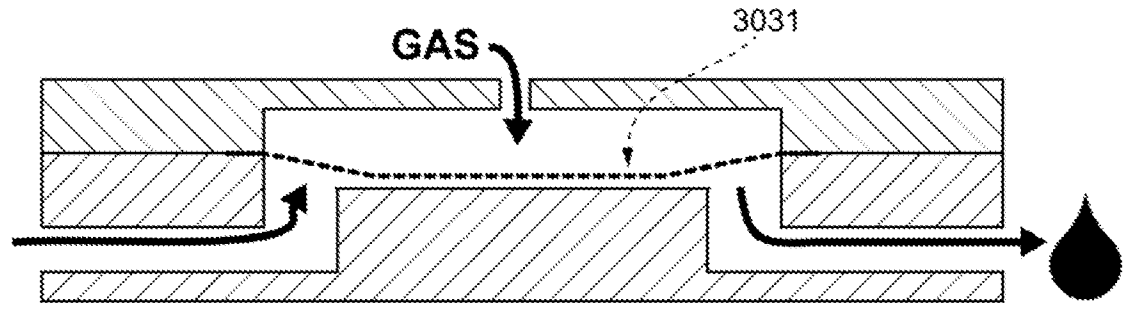


Fig. 29A



3020

Fig. 29B



3030

Fig. 30A

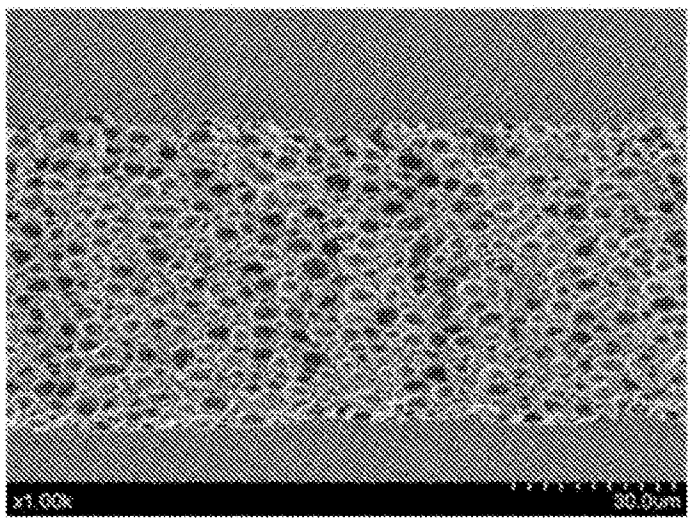


Fig. 30B

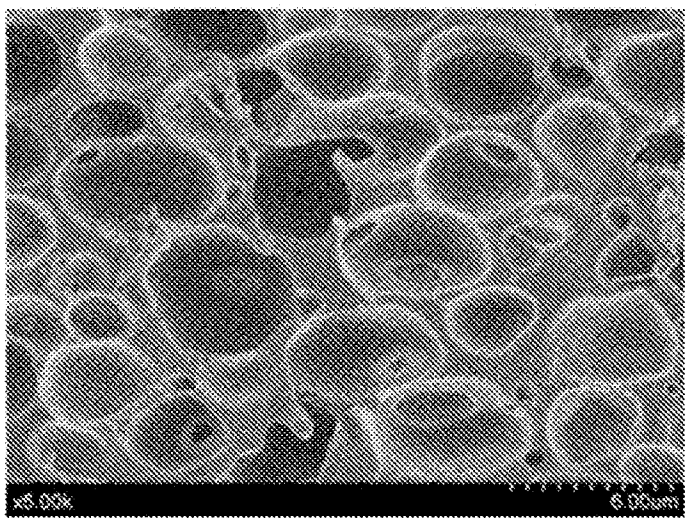


Fig. 30C

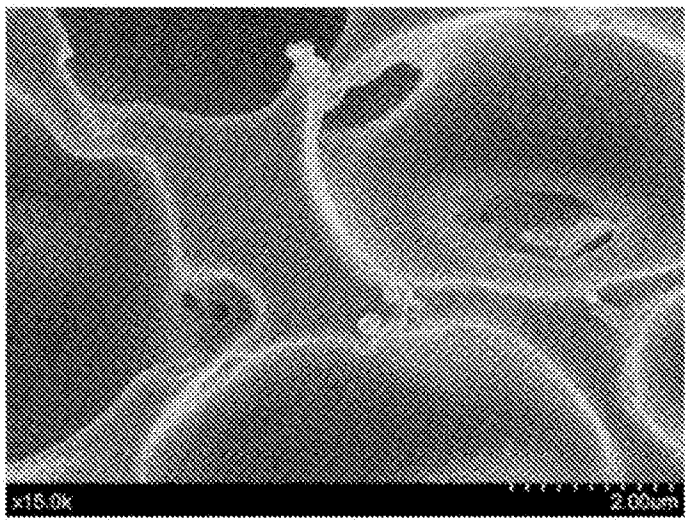


Fig. 31

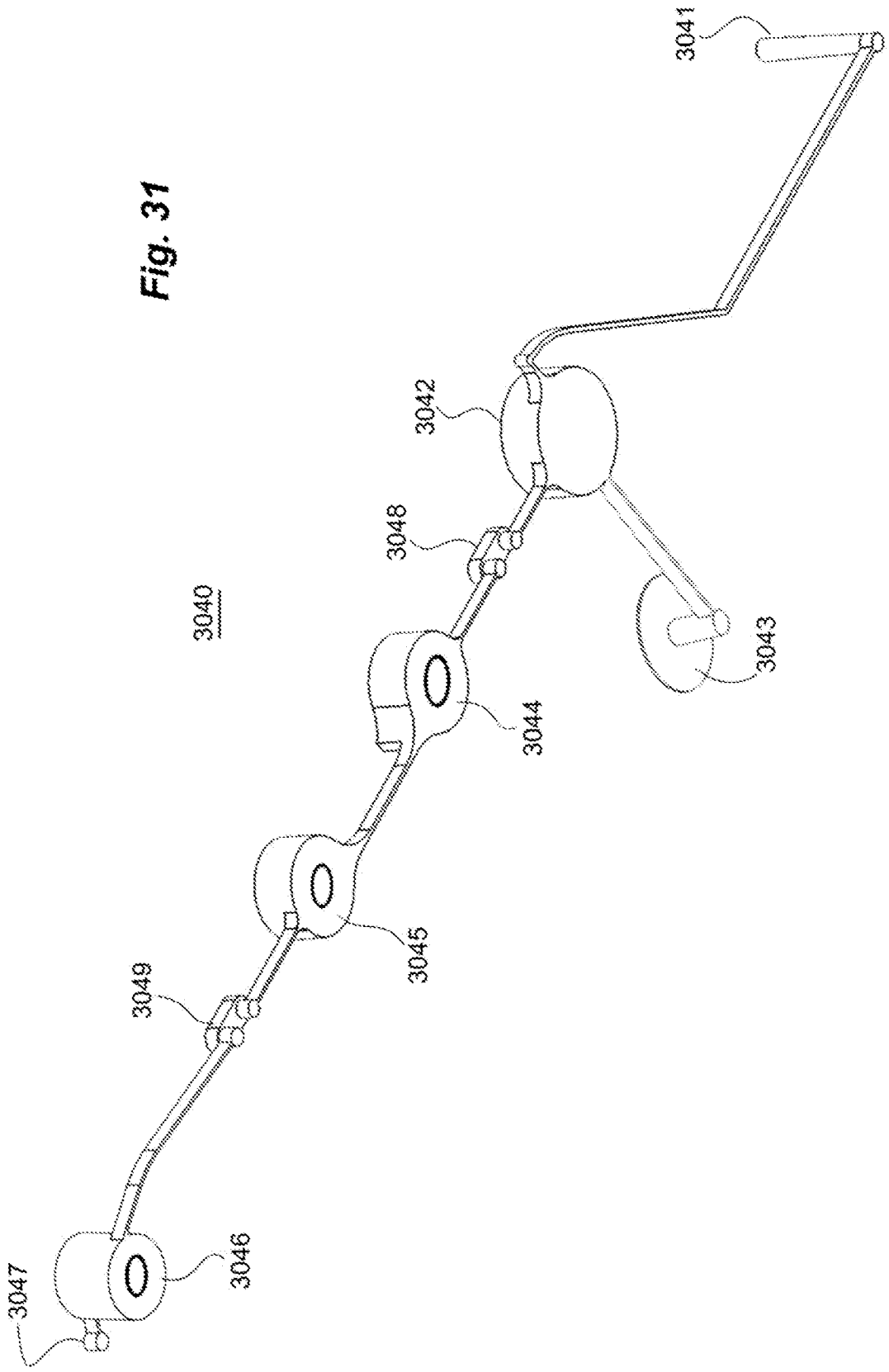


Fig. 32

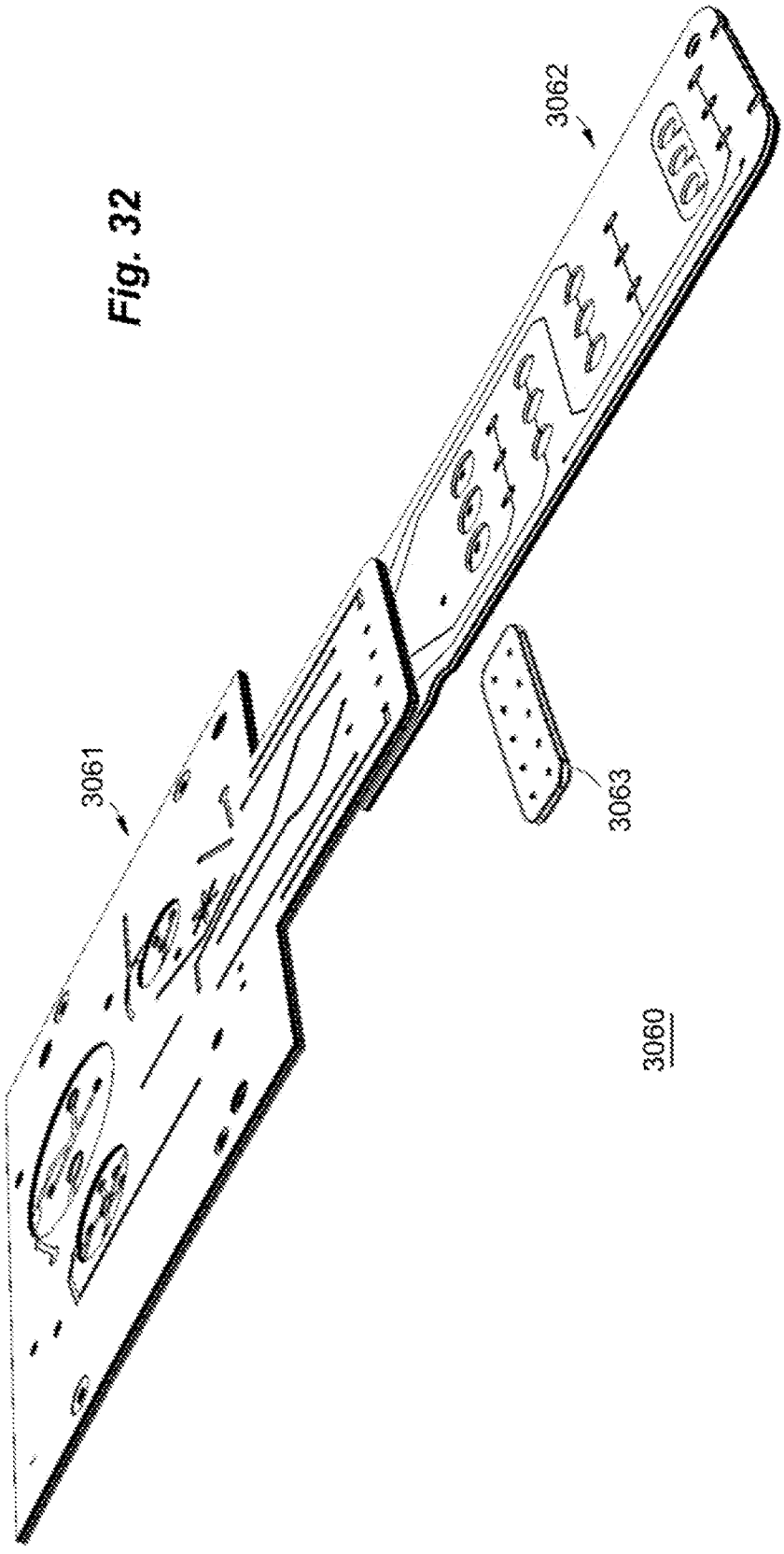


Fig. 33

POLYMER FILM	Yield Stress [7] (MPa)
Polyethylene Terephthalate (PET, biaxially oriented)[1]	116.0
Polyethylene Terephthalate (PET, biaxially oriented)[2]	99.9
High Density Polyethylene (HDPE)[3]	29.8 (26 - 33)
LDPE/EVA/PVDC/EVA/LDPE[4] co-laminate	15.4
Linear Low Density Polyethylenes	(1.8 - 20.3)
Low Density Polyethylene (LDPE)[5]	10.3 (3.5 - 20.0)
Polyvinylidene Chloride	(9 - 12)
Ethylene Vinyl Acetate Copolymers	(8 - 10)
Polytetrafluoroether, microporous[6]	2.2

1 - Mylar® 1 mil Dupont Teijin Films (Hopewell VA) determined here experimentally, Instron, RT, strain rate 150 mm/min, gauge length 1.5 cm between clamps

2 - Mylar® 2.5 mil, per ASTM std test (ASTM Intl, 2010. Publ D882-10 "Standard Test Method for Tensile Properties of Thin Plastic Sheeting", pp 1-10

3 - HDPE 1 mil per ASTM std test (ASTM Intl, 2010. Publ D882-10 "Standard Test Method for Tensile Properties of Thin Plastic Sheeting", pp 1-10

4 - Saranex 450® 1 mil (Dow, Newark DE) data determined here experimentally, Instron, RT, strain rate 150 mm/min, gauge length 1.5 cm between clamps

5 - LDPE 1 mil per ASTM std test (ASTM Intl, 2010. Publ D882-10 "Standard Test Method for Tensile Properties of Thin Plastic Sheeting", pp 1-10

6 - Mupor® 1 mil (Porex, Fairburn GA) data determined here experimentally, Instron, RT, strain rate 150 mm/min, gauge length 1.5 cm between clamps

7 - Parentheses indicate literature ranges