Video Article

Microfluidic Chips Controlled with Elastomeric Microvalve Arrays

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Abstract

Miniaturized microfluidic systems provide simple and effective solutions for low-cost point-of-care diagnostics and high-throughput biomedical assays. Robust flow control and precise fluidic volumes are two critical requirements for these applications. We have developed microfluidic chips featuring elastomeric polydimethylsiloxane (PDMS) microvalve arrays that: 1) need no extra energy source to close the fluidic path, hence the loaded device is highly portable; and 2) allow for microfabricating deep (up to 1 mm) channels with vertical sidewalls and resulting in very precise features.

The PDMS microvalves-based devices consist of three layers: a fluidic layer containing fluidic paths and microchambers of various sizes, a control layer containing the microchannels necessary to actuate the fluidic path with microvalves, and a middle thin PDMS membrane that is bound to the control layer. Fluidic layer and control layers are made by replica molding of PDMS from SU-8 photoresist masters, and the thin PDMS membrane is made by spinning PDMS at specified heights. The control layer is bonded to the thin PDMS membrane after oxygen activation of both, and then assembled with the fluidic layer. The microvalves are closed at rest and can be opened by applying negative pressure (e.g., house vacuum). Microvalve closure and opening are automated via solenoid valves controlled by computer software.

Here, we demonstrate two microvalve-based microfluidic chips for two different applications. The first chip allows for storing and mixing precise sub-nanoliter volumes of aqueous solutions at various mixing ratios. The second chip allows for computer-controlled perfusion of microfluidic cell cultures.

The devices are easy to fabricate and simple to control. Due to the biocompatibility of PDMS, these microchips could have broad applications in miniaturized diagnostic assays as well as basic cell biology studies.

Protocol

Microfluidic device design using CorelDraw or AutoCAD software

Principle of PDMS microvalves-based devices: The devices consist of three layers: a fluidic layer containing microchambers of various sizes, a "control layer" containing the microchannels necessary to actuate the fluidic path with microvalves, and a middle thin PDMS membrane that is bound to the control layer. At rest, due to the compliance and hydrophobicity of PDMS, the membrane seals (reversibly) against its seat, therefore the chambers remain isolated from each other without energy input. Valves can be opened by applying negative pressure (e.g., house vacuum), so the PDMS membrane deflects down and separates from the surface that supports the wall between two fluidic chambers, thus connecting the fluidic path. Valve closure can be achieved by switching the pressure setting from vacuum to atmospheric pressure.

Fluidic layer and control layer patterns were designed using CorelDraw or AutoCAD software. Masks containing these designs were printed at high resolution (8,000 to 20,000 dpi) on transparency films through commercial services (CAD/Art services, Bandon, OR) (masks not shown).

Fabrication of silicon masters using standard SU-8 photolithography

1. Standard SU-8 photolithography methods were used to create SU-8 "masters" (SU-8 2050, MicroChem, Newton, MA) for the microfluidic layer and the valve control layer in a cleanroom (*not shown in this video*).
2. To facilitate release, prior to PDMS replication the SU-8, masters were silanized by exposure to a vapor of a fluorosilane ((tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (TFOCS)), in a desiccator jar (without drying pellets) attached to a vacuum source. The desiccator chamber must be located inside a chemical fume hood owing to the corrosive nature of TFOCS vapors.
3. Place a small portion of absorbent paper towel inside the desiccator chamber. Add a drop of TFOCS to the paper towel and evacuate the air from the chamber. Apply vacuum for 1 min and turn off. Close the vacuum and allow 30 min for deposition. Keep the masters in closed containers for future use.

Replica molding of PDMS from the masters

1. The fluidic layer and the control layer are made by replica molding of PDMS from SU-8 masters.
2. Thoroughly mixing PDMS pre-polymer and cross-linker (10:1 wt. Ratio), de-bubble in a desiccator for 10-15 min until bubbles clear.
3. Cut silicone tubing into 1-2 cm long pieces. Choose the appropriate size of tubing according to application. We use 1.14 mm I.D. tubings here for easy connection to 1/16 inch O.D tubing later.
4. Use Duco® Cement to glue tubing onto the inlet regions of the SU-8 master of the control layer. Be careful not to use too much glue, as the silicone tubing is made of the same components as PDMS, and the tubing will be embedded into the PDMS microfluidic device, creating air and fluidic tight inlets/outlets.
5. In our device, inlet regions are designed on the masks of both the fluidic and the control layers, but silicone tubing inlets are molded only into one layer (for example, control layer) of the device. To create inlets to the fluidic layer, we manually remove or puncture the few sections of

the membrane that cover the inlet regions. Therefore, after alignment and assembly, all microchannels (those that carry flow as well as those that control the valves) are accessible from the top of the device so that the bottom surface is planar, enabling imaging of the device on a conventional microscope stage.

6. Carefully pour de-bubbled PDMS onto both masters, around the tubing in the control-layer master. De-bubble again in a desiccator. After de-bubbling is complete, put into 65°C oven for > 1 hour for curing.
7. Remove cured PDMS-covered masters from the oven.
8. Cut individual devices from the masters (each master contains three identical devices) and peel off.
9. Remove glue from the inlet regions using a needle or a pair of forceps.
10. Take the control layer PDMS into the cleanroom.

Thin PDMS Membrane Manufacturing

1. As shown in the device principle, the middle layer consists of a ~12 μm -thick PDMS membrane.
2. Mix 10:1 wt. ratio of PDMS prepolymer/curing agent mixture with hexane (3:1 wt. ratio) by vortexing.
3. Move into a clean room. (A dust-free environment is critical for ensuring that the PDMS membranes are free of defects; dust particles may result in membranes containing holes and/or defectively bound to the replica mold.)
4. Put a silanized 3 inch -diameter wafer onto the vacuum chuck of a Solitec spinner. The wafer has to be silanized (derivatized with fluorosilane) prior to PDMS spinning to facilitate the release of PDMS from silicon surfaces. The Teflon bowl outside of the chuck was wrapped with plastic film for easy cleaning.
5. Dispense 2-3 ml of PDMS/Hexane mixture onto the wafer using an 18-Gauge syringe needle (for minimizing bubbles).
6. Set spin parameters. Spin at 7000 rpm for 30 sec, resulting in a PDMS film of ~ 12 μm thickness.
7. Heat the wafer at 85°C for 4 min on a hot plate to cure the PDMS film.

Multilayer PDMS device bonding and assembly

1. Put the control layer and the PDMS membrane into an oxygen plasma chamber. Turn on plasma for 30 sec (oxygen pressure 30 psi, flow rate 3-5 SCFH, 550W). Bring the control layer into contact with the PDMS membrane immediately (within 5 minutes) after oxygen activation. System parameters, such as oxygen pressure, flow rate, and plasma power and treatment time, are empirically configured according to different applications.
2. Wait for 5 min, and remove the control layer from the wafer along with the membrane.
3. Remove membranes on the inlets areas so that both control and fluidic layers are accessible from the top via tubing.
4. Align the control layer (with tubings as inlets) with the fluidic layer (planar) under a stereoscope. Because PDMS seals onto PDMS, no permanent bonding is required.

Computer-controlled opening and closing of PDMS microvalves by vacuum or pressure

1. After device alignment and assembly, insert 1/16 inch O.D. (1/32 inch I.D.) Tygon tubing into the 1.14 mm I.D. silicone inlets and connect the inlets to the pressure sources or the fluidic reservoirs.
2. For opening and closing valves, pressures are controlled by a vacuum line and an air pressure line connected through two pressure regulators to an array of miniature three-way solenoid valves.
3. The solenoid valves are connected to National Instruments data acquisition hardware controlled via Labview software.
4. Device operation and membrane deflection are visualized with a color CCD camera (SPOT RT, Diagnostic Instruments, Sterling Heights, MI).

Parallel mixing of two different color dyes in different defined nanoliter volumes

We demonstrate the operation of a parallel mixer that allows for storing and mixing precise sub-nanoliter volumes of aqueous solutions at various mixing ratios:

1. The fluidic layer contains two arrays of microchambers: Along array A, the size of the microchambers decreases, starting from the left, from 200 μm x 400 μm to 200 μm x 40 μm ; A_{10} is a 500 μm x 40 μm chamber and is used just for fluidic connection in Array A; to the right of chamber A_{10} is a set of chambers symmetrically increasing in sizes. The chambers in array B are designed such that the added volume of any two adjacent chambers in different rows always equal to. A_0 , A_{0r} and B_{10} , B_{10r} are designed as respective controls for solutions A and B without mixing.
2. The control layer has two independently-controlled sets of valves. A set of valves $\{V_1\}$ is used to connect two chamber arrays with their respective inlets, whereas a second set of valves $\{V_2\}$ is used to connect each pair of chambers in the two arrays.
3. Fill the microchambers by opening valve set $\{V_1\}$ to allow flow of two dye solutions to arrays A and B, respectively. Flow of solutions can be achieved either by hand or by vacuum pulling controlled with solenoid valves. If air bubbles form in the microchambers, more solution can be pushed to remove the bubbles, or the device can be left for a few minutes and bubbles will disappear due to the air permeability of PDMS.
4. Close the valve set $\{V_1\}$ to isolate each chamber in both arrays.
5. Open valve set $\{V_2\}$ to allow fluid mixing between adjacent chambers in different arrays. Mixing only takes ~1-2 min to complete for these volumes.
6. Close $\{V_2\}$ to push the fluid back to each fluidic chamber and chambers deform back to their original shape. Since the two fluidic arrays are designed with chambers of 11 different sizes, 11 different mixing ratios are produced in a single mixing step.

An integrated microfluidic system for computer-controlled perfusion of microfluidic cell cultures

We demonstrate a microfluidic system that is capable of the automated perfusion of multiple solutions to a single cell culture chamber. The inlets are controlled by microvalves, which can be activated in any sequence of single inlets, various combinations, or all at once. The device is capable of producing gradients or mixtures of the various solutions.

This device also consists of three layers: a fluidic layer, a control layer, and a middle thin PDMS membrane.

Alternative fabrication steps for this device:

1. The inlet ports for the fluidic channels and control channels are "punched" using a 1.2 mm diameter Harris Micro-Punch (Ted Pella, Inc.). Tubing is connected to the inlets by using blunted 18 gauge needles which are inserted in to the PDMS through the control layer. This allows for a denser packing of inlets than the silicone tubing. The compliance of the PDMS provides a tight seal around the needles to effectively deliver fluid or pneumatic pressure.
2. As previously described, the bonding of the thin PDMS membrane to the control layer is accomplished using exposure to oxygen plasma.
3. The fluidic layer is prepared by replica molding with PDMS pre-polymer and cross-linker at a ratio of 5:1 and partially curing for 25 minutes at 60°C in a convection oven. At this point, the partially cured fluidic layer is still tacky, yet it can be removed from the master.
4. The fluidic layer is manually aligned to the pre-assembled control and membrane layers using a stereoscope. The assembled device is then placed on a hotplate for 5 minutes at 80°C. Next, the valve control lines are connected to the automated controller and the valves are actuated until the membrane detaches from fluidic layer at all the valve seats by applying vacuum. After "detaching" the valves, the computer controller is set to cycle the valves on and off while the device is further cured on the hotplate at 80°C for at least 1 hour.

The features of our integrated microfluidic system: The device is capable of automated perfusion of 16 different solutions to a cell culture chamber using a multiplexed valving scheme. The channel design ensures that the resistance of all the inlets is balanced. Our microvalve design isolates solutions and controls rinsing through integrated channels for the rapid removal of fluid, which limits cross-contamination. An integrated herringbone mixer can be activated to produce mixtures of different inlets. Additionally, there are four varying resistance channels that can be activated to alter the flow rate.

Discussion

Main advantages of our microvalve design:

1. No extra energy source is required to close the fluidic path, hence the loaded device is highly portable; and
2. The device can be built by PDMS replicas from photolithographically-patterned SU-8 molds, allowing for microfabricating deep (up to 1 mm) channels with vertical sidewalls (i.e. the height of the features can be specified independently of their width) and resulting in very precise features.

Advantages of the parallel mixer:

1. It is easy to fabricate and simple to control.
2. The volumes are photolithographically defined and, thus, very precise.
3. Fluid and reagents can be stored in the microdevice for several days, allowing for highly portable assays.
4. Notably, PDMS is biocompatible, so the device has broad applicability in miniaturized diagnostic assays as well as in cell-based assays such as drug screening and enzyme-based biomolecule detection.

Advantages of the integrated microfluidic perfusion chamber:

1. It is capable of the automated perfusion of multiple chemicals solutions to a single cell culture chamber.
2. The inlets are controlled by microvalves, which can be activated in any sequence of single inlets, various combinations, or all at once.
3. The device is capable of producing gradients or mixtures of the various solutions.

Main cautions for the fabrication processes:

1. A dust-free environment is critical during PDMS membrane fabrication, which ensures that the membranes are free of defects.

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