Video Article

Brain Slice Stimulation Using a Microfluidic Network and Standard Perfusion Chamber

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Abstract

We have demonstrated the fabrication of a two-level microfluidic device that can be easily integrated with existing electrophysiology setups. The two-level microfluidic device is fabricated using a two-step standard negative resist lithography process [1]. The first level contains microchannels with inlet and outlet ports at each end. The second level contains microscale circular holes located midway of the channel length and centered along with channel width. Passive pumping method is used to pump fluids from the inlet port to the outlet port [2]. The microfluidic device is integrated with off-the-shelf perfusion chambers and allows seamless integration with the electrophysiology setup. The fluids introduced at the inlet ports flow through the microchannels towards the outlet ports and also escape through the circular openings located on top of the microchannels into the bath of the perfusion. Thus the bottom surface of the brain slice placed in the perfusion chamber bath and above the microfluidic device can be exposed with different neurotransmitters. The microscale thickness of the microfluidic device and the transparent nature of the materials [glass coverslip and PDMS (polydimethylsiloxane)] used to make the microfluidic device allow microscopy of the brain slice. The microfluidic device allows modulation (both spatial and temporal) of the chemical stimuli introduced to the brain slice microenvironments.

Protocol

SU-8 mold fabrication

Master preparation

1. The SU-8 master on silicon wafer substrate is prepared using a two-step standard negative resist lithography process.
2. The alignment marks on the silicon wafer are removed using a razor blade as the height of these structures (located along the outer periphery of the wafer) is more than the actual device structures.
3. The silicon wafer is then cleaned using isopropyl alcohol and dried in a stream of N_2. Support pillars made out of tape with thickness less than the tallest device structure replace the alignment marks on four sides of the wafer.
   Note: If the support pillars height is more than the tallest device structures, the through holes are not formed in the PDMS mold.
4. The master silicon wafer is placed on a hot plate at the room temperature.

PDMS solution preparation

1. Four grams of polydimethylsiloxane (PDMS) solution is prepared by thoroughly mixing 10 parts of silicone elastomer with 1 part of curing agent.
   Note: Make sure that the two solutions are mixed uniformly to obtain similar physical properties throughout the PDMS mold.
2. The bubbles generated in the PDMS solution during the mixing process are removed using a vacuum desiccator.

PDMS coating and curing

1. The bubble-free PDMS solution is slowly dispensed onto the SU-8 master making sure that bubbles are not generated during the dispensing process.
2. One end of a write-on transparency film is then placed on the hot plate and slowly placed on the PDMS solution to evenly spread the PDMS onto the SU-8 master. Any bubbles generated during this process need to be removed using a probe.
   [Note: Do not lift the transparency to prevent generation of more bubbles.]
3. A borofloat slab is placed on top of the transparency sheet to apply uniform pressure. Gentle pressure is applied on the top surface of the tallest circular structures (located midway of the channel lengths and centered along the width of the channels) such that there is minimal or no PDMS sandwiched between the transparency and the SU-8 surface of the circular structures.
4. Three more borofloat slabs are placed on top of the silicon wafer-borofloat slab sandwich to apply constant pressure on the top surface of the circular opening structures before and during the curing process.
5. The temperature of the hot plate is then increased to 75°C and the PDMS is cured at this temperature for 1 hour. The hot plate is brought to a temperature of 50°C.
6. The slabs are removed and the transparency is gently removed leaving behind the master and the thin PDMS sheet coated on top of it.

Construction of the microfluidic device

Removal of PDMS sheet from master

1. The outer boundary of the perfusion chamber is carved out on the PDMS sheet using a razor blade after aligning the ports on the perfusion chamber with the ports on the SU-8 master.
2. The PDMS sheet is then gently removed from the master, pulling along the length of the channels to prevent tearing of PDMS. The PDMS sheet is placed on a transparency sheet with the microfluidic network surface facing upwards.
3. The inlet and outlet ports are then made using a cork borer.

Bonding of PDMS sheet to glass coverslip

1. The bonding surface of the PDMS sheet (the surface containing the microfluidic network) and a glass coverslip are then gently cleaned using a 3M scotch tape, placed on a sheet of transparency, and finally placed in the O_2 plasma chamber.
2. The bonding surfaces are treated with 165 Watts plasma for 10 s. The plasma treated surface of the glass coverslip is immediately bonded to the plasma treated surface of the PDMS sheet.
   Note: Apply gentle pressure on the glass surface to remove any air bubbles trapped between the bonding surfaces.
3. Allow 5 minutes to obtain good bonding between the PDMS and glass. After 5 minutes gently remove the transparency on which the PDMS sheet was placed for the bonding process.
4. The microfluidic device is placed in the oxygen plasma system to make the channels hydrophilic. The plasma treatment is done at 165 Watts for 1 minute.

Integration of microfluidic device and perfusion chamber

Preparation of the perfusion chamber

1. An off-the-shelf perfusion chamber was ordered, and inlet and outlet ports were drilled so that when the microfluidic device and the chamber are bonded, the ports on the chamber and the device are aligned. The bottom surface of the perfusion chamber is coated with PDMS to obtain a tight seal between the chamber and the microfluidic device.
2. A write-on transparency sheet is placed on a hot plate at the room temperature.
3. One gram PDMS solution is prepared in a process similar to that described earlier. The bubble-free PDMS solution is then slowly dispensed onto the transparency sheet avoiding generation of bubbles.
4. The perfusion chamber is then placed on the PDMS solution.
5. A borofloat slab is then placed on top of the chamber to apply uniform pressure and to obtain a thin coating of PDMS on the bottom surface of the chamber.
6. The temperature of the hot plate is then increased to 75°C and the PDMS is cured at this temperature for 1 hour.

Bonding of microfluidic device and perfusion chamber

1. The slab is removed and the transparency is gently removed leaving behind the perfusion chamber and the thin PDMS sheet coated on its bottom surface. Unwanted PDMS is removed using a razor blade and a sharp tip probe for PDMS removal from access ports.
2. The PDMS surface of the microfluidic device and the PDMS bottom surface of the chamber are then gently cleaned using a 3M tape, placed on a sheet of transparency, and finally placed in the oxygen plasma chamber.
3. The surfaces are treated with 165 Watts plasma for 10 s. The PDMS coated surface of the chamber is immediately bonded to the PDMS surface of the microfluidic device.
   Note: Apply gentle pressure on the glass surface to remove any air bubbles trapped between the bonding surfaces.
4. Immediately before the use, the device is placed in the oxygen plasma system to make the channels hydrophilic. The plasma treatment is done at 165 Watts for 1 minute.

Exposing brain slices to neurochemical microenvironment using the microfluidic device

1. The hydrophilic microchannels of the (microfluidic device)-(perfusion chamber) combination are filled with standard ACSF (Artificial Cerebral Spinal Fluid) solution.
2. Dispense small drops of ACSF solution at the inlet ports and allow the solution to be wicked up into the channels. Remove any bubbles left using a syringe from the outlet port. Dispense a large drop of ACSF solution at the outlet port to allow passive pumping of fluids from inlet to outlet ports of the microfluidic device.
   Note: Care should be taken to remove all bubbles from the channels to allow flow of fluids using passive pumping method.
3. Fix the (microfluidic device)-(perfusion chamber) combo in the plain platform and fix the plain platform into the microscope adapter.
4. Connect the standard inlet and outlet (suction) tubing to the perfusion chamber for continuous perfusion of the brain slice with standard ACSF solution. The ACSF solution is continuously aspirated with 95% O2-5% CO_2.
   Note: Adjust the position of the suction tubing to maintain constant level of ACSF in the bath of the perfusion chamber.
5. Once the perfusion chamber is filled with ACSF solution, place a brain slice in the perfusion chamber using a dropper. Using a probe, position the brain slice above the circular openings in the microfluidic device. Once the brain slice is at the desired position over the circular openings, use a slice anchor to immobilize the brain slice.
6. The microfluidic device can now be used to expose the brain slices (placed in the perfusion chamber and on top of the microfluidic network) to various neurotransmitters using passive pumping of fluids.

Disclosures

The authors are open to collaborations involving the demonstrated microfluidic technology to different fields of biology.

Discussion

Existing macroscale or microscale brain slice perfusion chambers are limited in terms of the spatial resolution they provide to expose brain slices with neurotransmitters. The microfluidic device technology demonstrated here overcomes this limitation using simple bioMEMS techniques. It is anticipated that the simplicity in the fabrication of the microfluidic device and the ease in integrating it with existing electrophysiology setups will allow widespread application of the demonstrated device technology. Interesting experiments that were not possible earlier can be performed with the current microfluidic device. Different microenvironments of the brain slice can be exposed to different neurotransmitters at different time scales.

The current prototype device comprises only four parallel channels and circular openings located next to each other. However, this device layout can be easily changed by implementing different designs that would have openings of different shapes or sizes, or the microchannels could be meandered in a fashion that would lead to the positioning of the openings in different microenvironments of brain slices.

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