Video Article

Fabrication of a Microfluidic Device for the Compartmentalization of Neuron Soma and Axons

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URL: https://www.jove.com/video/261
DOI: doi:10.3791/261

Keywords: Issue 7, Cell Biology, Biomedical Engineering, Neuroscience, Cell Culture, Axonal Regeneration

Date Published: 8/22/2007

Citation: Harris, J., Lee, H., Vahidi, B., Tu, C., Cribbs, D., Jeon, N.L., Cotman, C. Fabrication of a Microfluidic Device for the Compartmentalization of Neuron Soma and Axons. *J. Vis. Exp.* (7), e261, doi:10.3791/261 (2007).

Abstract

In this video, we demonstrate the technique of soft lithography with polydimethyl siloxane (PDMS) which we use to fabricate a microfluidic device for culturing neurons. Previously, a silicon wafer was patterned with the design for the neuron microfluidic device using SU-8 and photolithography to create a master mold, or what we simply refer to as a "master". Next, we pour the silicon polymer PDMS on top of the master which is then cured by heating the PDMS to 80°C for 1 hour. The PDMS forms a negative mold of the device. The PDMS is then carefully cut and lifted away from the master. Holes are punched where the reservoirs will be and the excess PDMS trimmed away from the device. Nitrogen is used to blow away any excess debris from the device. At this point the devices are now ready for use and can either bonded to corning No. 1 cover glass with a plasma sterilizer/cleaner or can be reversibly bound to the cover glass by simply placing the device on top of the cover glass. The reversible bonding of the device to glass is covered in a separate video and requires first that the device be sterilized either with 70% ethanol or by autoclaving. Plasma treating sterilizes the devices so no further treatment is necessary. It is, however, important, when plasma-treating the devices, to add liquid to the devices within 10 minutes of the plasma treatment while the surfaces are still hydrophilic. Waiting longer than 10 minutes to add liquid to the device makes it difficult for the liquid to enter the device. The neuron devices are typically plasma-bound to cover glass and 0.5 mg/ml poly-L-lysine (PLL) in pH 8.5 borate buffer is immediately added to the device. After a minimum of 3 hours incubating with PLL, the devices are washed with dH2O water a minimum of 3 times with at least 15 minutes between each wash. Next, the water is removed and fresh media is added to the device. At this point the device is ready for use. It is important to remember at this point to never remove all the media from the device. Always leave media in the main channel.

Video Link

The video component of this article can be found at https://www.jove.com/video/261/

Protocol

Part 1: Preparing the microfluidic neuron device

1. A 3" silicon wafer with a pattern made out of SU-8 by photolithography is used to cast the Polydimethylsiloxane (PDMS) microfluidic mold. We refer to the SU-8 patterned silicon wafer as master molds, or "masters".
2. PDMS is mixed with curing agent at a w/w ratio of 10:1, that is, for 10 grams of PDMS, 1 gram of curing agent is added to it.
3. The PDMS is then mixed well and poured onto the silicon wafer. The silicon wafer is contained within a 10 cm polystyrene Petri dish. It takes approximately 12 g to 15 g of PDMS to cover the master with a thickness of approximately 4 mm.
4. The master with the PDMS is then placed in a vacuum dessicator with the lid off the Petri dish, and vacuum is applied. This is done to help remove air bubbles from the PDMS that were introduced during the stirring in of the curing agent. It takes approximately 15 minutes for the air bubbles to be removed.
5. The master with PDMS is removed from the dessicator. An air gun with a 0.45 um filter is used to remove any remaining bubbles.
6. The master is then placed on an 80°C hot plate for 1 hour to cure.

Note: If a vacuum dessicator is not available, the master can be left at room temperature for several hours. The air bubbles will eventually dissipate on their own. If a hot plate is not available, the PDMS will cure at room temperature after 24 hours.

Note: It is also important to make sure that the master is level during the curing process.

Part 2: Cutting out the neuron microfluidic device

1. Once the PDMS is cured on the master, it is taken to a clean hood.
2. Using a surgical blade, a circle is cut around the perimeter of the devices. When one inserts the blade into the PDMS, it is important to make contact with the silicon wafer but to NOT put too much pressure on the wafer, else the master will crack.
3. With a circle cut all the way around the devices (there are 4 devices per each 3" Silicon Master), the PDMS is carefully lifted off the master. This can be started by gently twisting the surgical blade as it is inserted into the prior cut.
4. Next, reservoirs are punched into the device using an 8 mm tissue biopsy punch.
5. The devices are then quartered and excess PDMS trimmed away so that the device will fit neatly on Corning Cover Glass (No. 1) 24 mm x 40 mm. Nitrogen air is used to blow away any excess debris. Stubborn debris can also be removed using 3M Scotch Brand 471 vinyl tape.

Part 3: Plasma bonding the devices to glass cover slips.

1. Plasma Cleaner is used to bond A Harrick the neuron devices to the cover slips. Briefly, the devices are placed in the plasma cleaner and a vacuum pump is used to evacuate air from the chamber.
2. After the vacuum pressure has reached 300 milliTorr, power is turned on to the electrical coil and set to high.
3. The electrical coil creates plasma, "a partially ionized gas consisting of electrons, ions and neutral atoms or molecules" - http://www.harrickplasma.com/plasma_physics.php, out of the remaining air molecules left in the chamber. The plasma creates reactive species on the surfaces of the glass and the device, which when placed together will form a permanent bond. The plasma also turns the surfaces hydrophilic, which helps facilitate the addition of liquids. Additionally, the plasma cleaner sterilizes the devices.
   The surface of the PDMS device that contains the microfluidic device is placed face up into the plasma cleaner along with the glass slides. The plasma treated surfaces of the glass and microfluidic device are assembled in contact with each other at a clean bench, then placed in sterile 60 mm dishes. The treated surfaces form a tight irreversible bond.
4. Within 10 minutes of plasma bonding the devices to glass, Poly-L-Lysine (PLL) is added to the device. After 10 minutes, the hydrophilicity of the device will decrease making it difficult for the PLL to enter the device.
   Note: It is important to check for air bubbles in the device after the addition of PLL. Air bubbles in the main channel are undesirable, as they would oxidize cells. An easy method to remove any existing air bubbles is to use a P200 pipet filled with 150 ul of liquid (in this case PLL), place the tip directly at the opening of the main channel, and depress the P200 forcefully. This may need to be repeated several times but eventually the force from the pipet will drive the bubbles out.
5. The devices containing PLL are allowed to incubate overnight in a standard tissue culture incubator at 37°C with 5% CO_2.
6. The next day, the devices are rinsed twice quickly with autoclaved dH2O. During this process, liquid is never entirely removed from the main channels, only from the reservoirs. Removing liquid from the main channels will introduce bubbles. After 2 quick rinses, the devices are filled with autoclaved dH2O and placed back into the incubator for a minimum of 3 hours, or overnight.
7. The next day, the devices are once again rinsed 2 times quickly with autoclaved dH2O. Then, neural basal media, containing the necessary supplements B27 and glutamax for culturing primary rat neurons, is added to the devices. The devices are placed back in the incubator for future use. The devices are now ready for the seeding of neurons.

Discussion

In this video we have demonstrated how to make and prepare a PDMS microfluidic device that we use to culture neurons in. The device allows us to obtain a pure axonal fraction separated by microgrooves from the compartment containing a mix of cell bodies, dendrites, and axons. These devices allow the researcher to study the effects of various treatments as well as provide a platform to carry out physical injury on the axons and observe what effects these treatments have on the neurons. The device also provides a level of order to culturing neurons that helps facilitate transport studies. In addition, the microgrooves, which separate the cell culture compartment from the axon compartment, allows for fluidic isolation between the two compartments making it possible to treat one side of the device without effecting the other side of the device directly with the treatment. This property allows the researcher to study effects such as signal transduction and transport that result from the treatment.

References

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