PDMS Microfluidic Devices at SNF

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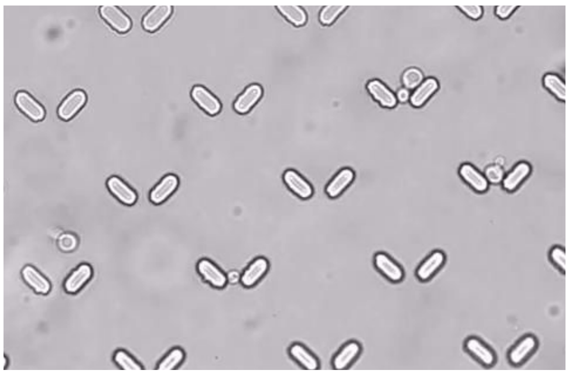
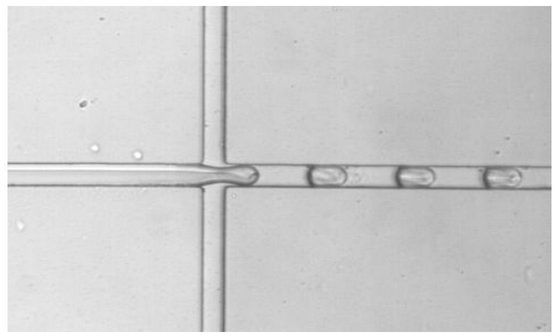
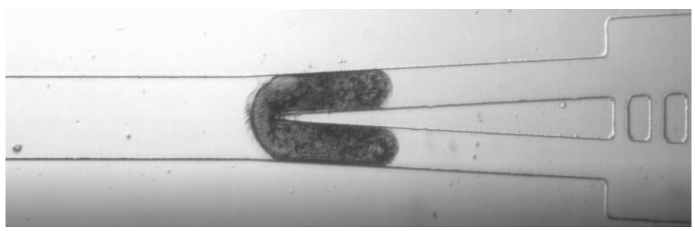






Introduction:

This is a general but detailed protocol for creating microfluidic devices out of PDMS (Sylgard 184) using the tools available at SNF. This protocol will work for most applications and has been developed over 4 years in Prof. Sindy Tang's microfluidics lab in at Stanford.

Please read all sub-points in the protocol in order to fully understand the intricacies of working with this seemingly easy to use material! :)

Protocol assumptions:

This protocol assumes the user has first created a microfluidic device master out of a suitable photoresist (such as SU-8) on a 3" or 4" silicon wafer, with the wafer inside either a 3" or 4" petri dish, respectively. The protocol also assumes that the microfluidic device master has been properly silanized to make the surface hydrophilic such that the PDMS can be easily peeled off the surface of the master. There are two other alternatives to silanizing: 1) The YES oven can also be used to deposit HMDS on the master prior to pouring PDMS. 2) The Drytek2/4 or MRC etcher can coat the master with fluorinated polymer.

Materials for making and using microfluidic devices

Sylgard 184 (PDMS) Base	Oven set to 65 ºC	BD 3 ml syringe (or equivalent)
Sylgard 184 Curing Agent	Plasma cleaner	Glass slides
Thinky mixer and Thinky mixer cups (or plastic spoon and dessicator)	1 mm Miltex Biopsy punch with plunger	Mass balance, with the scale covered in tin foil and placed on top of tin foil
PE Tubing (Scientific Commodities BB31695-PE/4)	BD 21G PrecisionGlide needles	Scalpel

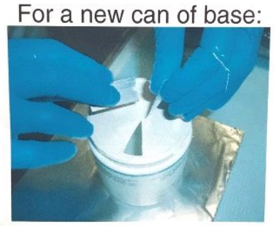
Protocol

1. Prepare PDMS for pouring

For a new container of PDMS base or curing agent, use a scalpel or razer blade to create pouring and venting holes.

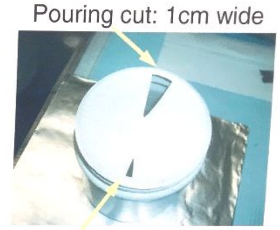
Create smaller versions of the same holes for the curing agent as well.

For a new can of base:



Make pouring and venting cuts

Pouring cut: 1cm wide



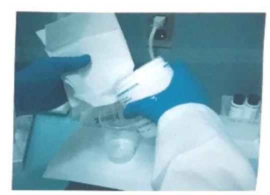
Venting cut: smaller

2. Pour PDMS components in cup

For this protocol, we will use a 10:1 PDMS base to curing agent ratio, which is the most common. The stiffness of the PDMS can be increased by using more curing agent or decreased by using less curing agent.

1. On the mass balance, tare the Thinky mixer cup.
    - ❖ The top of the mass balance, where the mass is weighed, should be covered with foil. The balance should be put on top of foil to guard against spills. Do your best to wipe up any spills after you're done with the balance.

Pour the base



Wipe the PDMS base off the rim of the can!

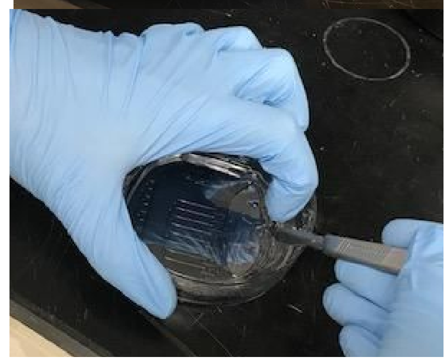
2. Tare the balance with the mixing cup on it, then pour and weigh the Sylgard 184 Curing Agent. The mass should be 1/10 the mass of the Sylgard 184 Base. Clean the outside of the container with a Kimwipe after use, without letting any particles enter into the container.
    - ❖ For the first PDMS pour on a 3" wafer, 25 – 30 g of PDMS should be used. Subsequent pours for later device replicas can use between 15 – 17 g. For the first PDMS pour on a 4" wafer, 44 – 53g of PDMS should be used. Subsequent pours for later device replicas can use around 26 – 35 g.
    - ❖ When weighing out PDMS, assume an unrecoverable mass of PDMS in the cup of 5 g. It is unrecoverable because it gets stuck to the cup when pouring.
    - ❖ Ideally, subsequent pours should not go above the height of the previously poured PDMS.
    - ❖ The PDMS should have a height of between 0.4 – 0.6 cm, as a sufficient height helps with plugging in the tubing when using the device.
    - ❖ Once mixed, the PDMS should be poured into the device mold within about 2 – 3 hours.
3. Mix and degass the PDMS using the Thinky mixer

- ❖ Rotate the counter balance knob until the indicator reads the TOTAL mass of the material (base + crosslinker) plus 40g. Note that the counter balance indicator numbers start at 40g.
- ❖ Slide close the instrument cover and start the recipe. The default recipe is recipe 5, which consists of 2 minutes of mixing and 2 minutes of defoaming. Once the recipe is complete, the PDMS can be directly poured onto a wafer for spin coating or in a mold before subsequent degassing.
- ❖ The maximum mass the Thinky mixer cups can hold is 140g.
- ❖ PDMS can also be mixed by hand using a clean plastic spoon. The PDMS will have a lot of bubbles in it but can be poured into the master mold and dessicator for 1 hour to remove the bubbles, like in the next step.

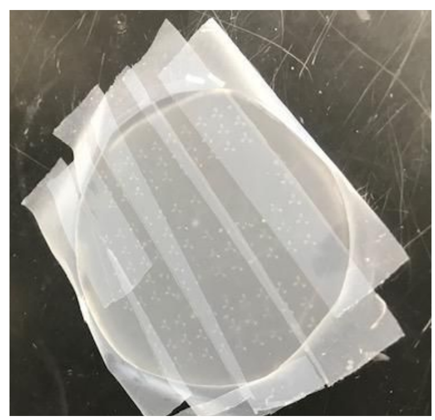
4. Put the device master onto the scale and tare it. From the mixed and degassed PDMS cup, slowly pour and weigh the required amount of PDMS into the device master (see notes in step 2). If any bubbles form, put the device master with PDMS into the dessicator for 30 min to 1 hour.
   - ❖ Don't leave the device in the dessicator for longer than 8 hours, or it can break the silicon wafer as it cures under pressure.
   - ❖ SNF guidelines state that PDMS should not be thrown in the trash before being cured (24 – 48 h at room temperature). After pouring the PDMS from the cup, please leave the cup and mixing spoon (if used) in the fume hood until it is cured before throwing it away.
5. Put the device master with PDMS into the oven at 65 ºC for a minimum of 1.5 hours but ideally at least 4 hours.
   - ❖ The PDMS will be mostly cured after 1.5 hours, but will continue to cure over time, making its mechanical properties unstable. This is particularly annoying when using a hole-punched device, as the holes will be too small for the tubing. Curing for at least 4 hours solves this problem, and is highly recommended.
   - ❖ If you are in a rush to make a lot of devices, you can initially cure each device for 1.5 hours, cut out the PDMS from the master, put tape of the device side of the PDMS, and

put it back in the oven for an additional 2 – 3 hours. This allows you to pour PDMS for an additional device.

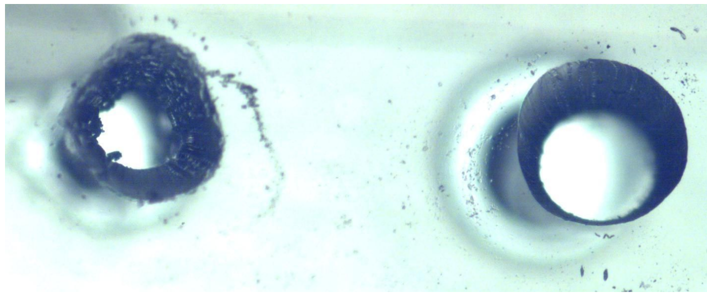
3. Post-cure PDMS device preparation and hole punching



1. Once the PDMS is cured, use a scalpel to carefully cut around the outside of the silicon wafer (but on the wafer itself).
2. Using the edge of the scalpel or a similar tool, slowly and carefully lift the edge of the PDMS off of the wafer. Once it gets high enough off the surface of the wafer, use your hand to slowly peel it off the rest of the way.
   - ❖ Small features can be better preserved by first putting a ml of so of ~70% ethanol in water on the top of the PDMS near where you will be pulling it off the wafer. When pulling the PDMS off the mold, the ethanol decreases the surface tension between the PDMS and the device mold, preventing the PDMS from sticking. If doing this step, use a clean-room quality air blower to get the ethanol off the PDMS and device mold before proceeding to the next step
3. Use clear scotch tape to cover the entire underside of the PDMS containing the channels, so that they don't get covered in dirt.
   - ❖ PDMS can be stored like this for months or years. Just make sure to clean the outside with tape before using to remove any dust buildup.



4. Put the PDMS device onto a soft surface, such as a silicone sheet or a spare piece of PDMS.
   - ❖ We use a large refrigerator magnet with a soft back in my lab, but PDMS works equally as well. The goal is to not let the hole puncher get dull, as it will create holes in the PDMS that are smaller and dirtier. This makes it harder to get the tubing into the holes when running the device and also can let PDMS junk enter the channel.
5. Using a 1 mm hole puncher (**Miltex Model: 33-31AA-P/25**), punch holes all the way through the PDMS at the inlet(s) and outlet(s) of the device. Push the plunger so that the punched PDMS exits the hole punch through the tape side of the PDMS device. You can lift the PDMS slightly when doing this to help the PDMS punch exit through the tape side.



Left: Bad hole punch. Right: Good hole punch

- ❖ It is a good idea to create a test hole first before punching all the inlets and outlets of your device. The hole should be round and without jagged edges. If not, you should use a new hole punch.
- ❖ A 1 mm hole puncher is good for use with tubing that is slightly bigger, at 1.22 mm outer diameter (OD). This is because the larger OD tubing can stretch the hole when plugging it into the device

6. Clean up all the little PDMS punches and replace the tape on the PDMS device.

4. PDMS device bonding with the oxygen plasma cleaner

The plasma etcher can be used to modify the surface chemistry of PDMS and other materials prior to bonding. This modification can be used to promote permanent bonding between devices and substrates. Common materials that are treated in this instrument are PDMS-PDMS, PDMS-glass, silicone-glass, and PDMS-silicon surfaces

1. Clean the surface of the glass slide or PDMS you will be bonding your device to.
    - ❖ The easiest way to do this is with clear scotch tape, and usually gives very good results. Glass slides can be cleaned better using acetone and isopropanol, followed by drying with a cleanroom quality air blower. Some people also use diluted Windex to clean glass slides. If your channels are smaller than 30 x 30 μm or so, then acetone and isopropanol might give more consistent results, but I recommend trying tape first.
    - ❖ Don’t use acetone on PDMS, as it will degrade it. Tape is sufficient.
2. If you haven’t already after hole punching, clean the device side of the PDMS device with new tape. If the top side of the PDMS is dusty, use tape on that side too.
3. Put the glass slide and the PDMS, device side facing up, into the plasma cleaner
4. Run the plasma cleaner in either SNF or SNSF (see operating settings below)
    - ❖ SNF Plasma Cleaner is closer to SNF, but requires a dummy process in order to set up the recipe
    - ❖ SNSF Flexible Cleanroom doesn’t require a dummy process to set up the recipe, and may be easier to use as a result
5. Remove the glass slide from the plasma cleaner. Remove the PDMS from the plasma cleaner and slowly put it onto the glass slide, device side down. You will see the PDMS start to bond as it makes contact with the glass slide (or PDMS if you are bonding to PDMS). You can help the PDMS bond by lightly tapping on the locations where it has not yet bonded so that it can make full contact.
    - ❖ The bond should be completed within a maximum of 5 minutes, but ideally as soon as possible.

6. Put the bonded device onto a hotplate set around 130 ºC for at least 5 minutes, or into the oven at 65 ºC overnight. This helps complete the bonding step.

SNF Plasma Cleaner (Allen Room 155A)

In order to use the SNF plasma cleaner, you must first be trained by Carsen Kline (carsen@stanford.edu).

ALWAYS RUN A DUMMY PROCESS WITH THE SNF PLASMA CLEANER!

A good starting recipe for a permanent bond is:

Gas type: oxygen, 15 sccm
RF Power: 35 W Forward, 0 W Reflected
Plasma Time: 30 seconds.
Vacuum Set Point: 250 mTorr

SNSF Flexible Cleanroom Plasma Cleaner

In order to use the SNSF plasma cleaner, you must first be trained by Tom Carver (carver@stanford.edu) and become a qualified user of the SNSF Flexible Cleanroom. To do this, you need to follow each of these steps in the order as listed here:

1. complete the process to become lab member of SNSF (Note: this includes establishing a valid Badger account)
2. review General Cleanroom Manual
3. review and complete the Flexible Cleanroom User Agreement (Note: this includes completion of online safety training modules through EH&S)

A good starting recipe for a permanent bond is:

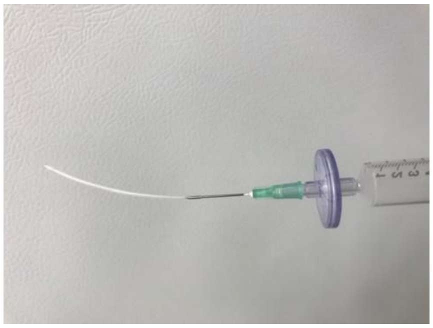
Gas 2 (oxygen): 2
RF Power: 80 W
Plasma Time: 15 - 30 seconds.
Plasma Type: Direct (ask Tom Carver if you don't know what this means)

ADDITIONAL CONSIDERATIONS WHEN USING MICROFLUIDIC DEVICES

Importance of channel surface hydrophobicity

Normally PDMS is hydrophobic. However, after plasma bonding PDMS becomes hydrophilic due to the creation of -OH groups on the surface, with the effect decaying to baseline over about 24 hours. This change in surface hydrophobicity can greatly affect the performance of many devices, especially if anything (such as cells) are touching the channel walls. Usually, devices should be used 24 hours after bonding in order to have a consistent surface hydrophobicity between experiments.

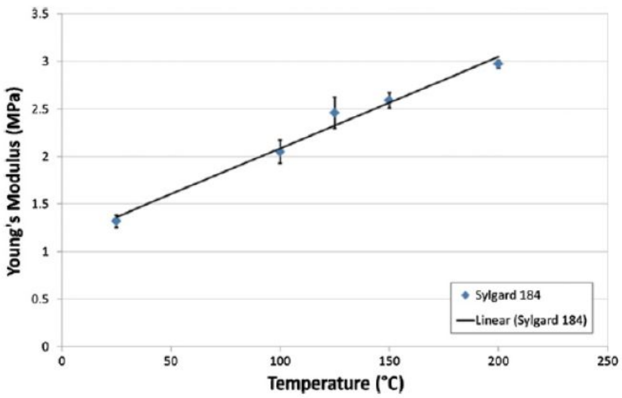
Hydrophilic channels can be created by putting an aqueous solution (such as DI water or PBS) into the channel immediately after bonding. The channel should be used within one or two days.



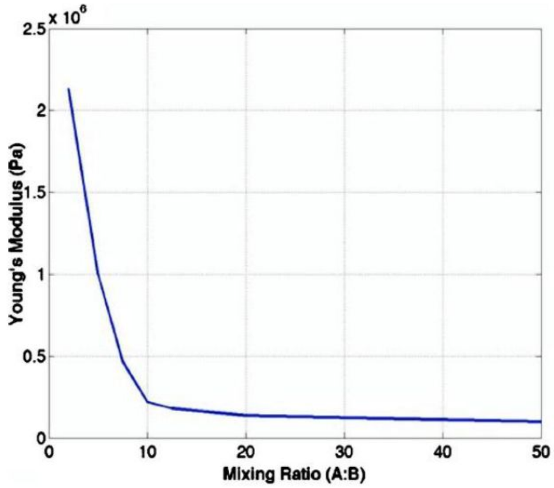
While PDMS is naturally hydrophobic, more hydrophobic channels can be created using Aquapel (http://www.aquapel.com/). First crack open the Aquapel container and let it drain into a 20 ml glass vial. This chemical should be pumped into the channel through a syringe with a 0.45 µm filter at the end. Afterwards, use another air-filled syringe with a filter to get the Aquapel out of the channels, and clean it up with a Kimwipe. The channel should then be put on a hotplate at about 150 ºC for at least 1 hour to evaporate the Aquapel. Channels do not normally need to be Aquapelled again after use, and it stays for a long time. The glass vial of Aquapel can be stored in the fridge after opening and is good for up to 1 month (or before visible precipitates form).

Controlling PDMS elasticity

Tensile strength vs curing temperature (10:1 base/curing agent ratio)



Tensile strength vs mixing ratio



Additional materials for making microfluidic devices

Sylgard 184 is the most commonly used form of PDMS but many different types of PDMS exist, each with its own advantages. For example, Sylgard 182 has a longer cure time and is very useful for spincoating thin (<1 mm) layers of PDMS, which can be useful for creating valves or other moving parts with air pressure through a channel. SNF has a PDMS spincoater which can be used for this purpose but is outside the scope of this protocol. Other PDMS varieties can be used which are much stiffer than Sylgard 184.

There are many different ways to create microfluidic devices, including Si or glass etching, hot embossing of plastic, micro-CNC milling, 3D printing (not recommended for small channels), laser-cut acrylic (not recommended for small channels), and injection molding (great for commercial processes but very high initial cost). Arguably PDMS is the easiest material for academic use, and works great for most applications.

Other materials similar to PDMS, but are stiffer, can be found in this nice review article: Lab on a Chip 11(22):3752-65

Additional master mold methods for making PDMS microfluidic devices

While SU-8 photoresist on a silicon wafer is the most commonly used master mold for PDMS microfluidic devices, other methods may also be used and have advantages for several applications. At SNF we have access to several exciting tools to make masters.

Nanoscribe

The Nanoscribe is essentially a 3D printer for photoresist with < 1 μm resolution that can be used to make microfluidic device master molds. This extremely powerful tool can be used to make PDMS microfluidic devices with gradients of height and complex 3D geometries. If you are interested in using the Nanoscribe, please find the protocol for using the Nanoscribe for making microfluidic devices on the SNF website and contact Swaroop Komerra (skommera@stanford.edu).

The protocol can be found here:

https://snfexfab.stanford.edu/nano/documentation/nano-nugget/stanford-nanoscribe-operating-procedure

Solidscape 3D Wax Printer

The Solidscape 3d Wax Printer is an extrusion printer capable of printing wax molds with 6 um precision and a minimum feature size of 200 um. Contact Swaroop Kommera for training (skommera@stanford.edu). A detailed protocol for using the printer as a mold for PDMS device fabrication can be found here:

https://docplayer.net/35329026-3d-printing-using-the-solidscape-studio-for-rapid-prototyping-of-optics-and-microfluidic-devices.html

Additional information can be found here:

https://docs.google.com/document/u/1/d/e/2PACX-1vTXzDYACWqT5gS0UAWbWSCxPD22pNm_A2wcnnT7bVje7r3Cw5f3hj97nnWJreQ-hTM8wKNDnaHGIdbI/pub#h.csow0yw9umhw

Heidelberg

The Heidelberg is a direct patterning device for spincoated and pre-baked photoresist. Currently in 2019 SNF only has a Heidelberg that can't be used with SU-8, but has plans on getting another Heidelberg for use with SU-8. The current 2019 Heidelberg can be used with MrDWL, which can be a suitable SU-8 replacement. If you are interested in using the Heidelberg, please contact Swaroop Komerra (skommera@stanford.edu).

Information and manuals on the Heidelberg can be found here:

https://snf.stanford.edu/SNF/equipment/nSiL/heidelberg-mla-150

Form2 3D Printer

The Form2 3D Printer is a new option for rapid prototyping of devices in SNF. Typically 3D printed parts inhibit the curing of PDMS and need to be surface-treated prior to use as a mold. If you are interested in doing this, please contact Swaroop Komerra (skommera@stanford.edu). Here is a starting protocol for using Form2 parts for PDMS casting:

1. Let the 3D printed mold soak in deionized water for 2 h
2. Cure in oven at 85-90°C for 18 h
3. The surface will be covered with a thin oily film, wash this away with dish washing detergent and deionized water.
4. Dry the mold and it is ready to use for PDMS casting.

More information on the Form 2 3D printer can be found here:

https://formlabs.com/3d-printers/form-2/

https://snfexfab.stanford.edu/equipment/training/form2-3d-printer-training