MICROFLUIDICS: FABRICATION and ASSEMBLY

Stocker Lab Protocol

Effective Date: 9 December 2013

1. Scope

Microfluidic channels may be fabricated by a variety of methods. One of the simplest and easiest is "**soft lithography**" (Xia & Whitesides 1998), of which some key steps are described below. This method relies on the casting of a liquid polymer (**polydimethylsiloxane** or **PDMS**) into a rubbery microchannel device. The mold for this casting is fabricated by depositing a pattern of hardened plastic (**SU-8**, Microchem) onto a silicon wafer via **photolithography** (commonly used for microelectronics fabrication). The positive relief of the SU-8 on the atomically flat wafer defines the height of the microchannels, while a transparent photo-mask defines the planar shape of the devices during the lithography step.

These molds (supplied by a lab member or MIT) are then filled with liquid PDMS, which conforms to the SU-8 relief and is subsequently hardened/cured. When removed from the mold, the PDMS slab will have the micron-scale geometry of the mold imprinted in it. This PDMS structure is then capped by a standard microscope slide or cover slip, which forms the final wall of the channel. The resulting microchannel may then be mated with tubing and connected to a syringe pump for control.

2. Definitions/Acronyms

PDMS	Polydimethylsiloxane
OD	Outer Diameter
EtOH	Ethanol

3. Personal Protective Equipment

Lab coat, gloves, and eye protection shall be worn. The curing agent has an ingredient that is considered a carcinogen so be sure to avoid skin contact.

4. Materials and Equipment

4.1. Casting PDMS Channels

- Master Mold mounted in a plastic petri dish
  SU-8 on Silicon wafer (provided by a lab member or MIT)

- Sylgard 184 Silicone Elastomer Kit (PDMS)
  Dow Corning – includes a base and curing agent
- Balance (w/ 0.1g resolution)
- Vacuum pump
- Desiccator
- Hotplate
- Disposable plastic cup
- Plastic knife
- Canned air (“Aeorduster” or “Whoosh”)

4.2. Removing PDMS Channels from Mold

- Razor Blade (Sharp and Clean)
  Exacto knives
- Harris Uni-core Punch with a diameter equal to the OD of the channel tubing.
  Ted Pella Inc.; 1mm provided by MIT or Miltex (Biopsy punch)
- Cutting Mat

4.3. Bonding PDMS Channels to Glass

- Glass slide or Cover Slip
- EtOH (70-100%)
- Canned air
- High Frequency Coil/ Plasma Generator
  Electro-Technic Products BD-20

NOTE: Ensure all materials and equipment are clean and dust free.

5. Preparation

5.1. Casting PDMS channels is very messy so you should designate a space for this and cover the area with a plastic liner to prevent PDMS from ruining cabinetry.

6. Procedure

6.1. Casting PDMS Channels

6.1.1. Estimate the approximate volume of PDMS needed to fill the master mold to the desired depth (typically ~2-5 mm). Then convert that volume to mass (PDMS density= 1.11g/ml). For the 4" wafer molds, this should be in the range of 10-15 g per mold.

6.1.2. Place the plastic cup on the scale and tare/zero.

6.1.3. Weigh out a 10:1 batch of base to hardener. Add the hardener before the base. The base is very viscous so it is best to use a large syringe (e.g. ~60ml) with the tip cut off.

6.1.4. Stir vigorously for ~ 1-2min until the solution is thoroughly mixed. This process will naturally entrain bubbles making the solution frothy indicating that the solution is well mixed. Use the flat side of the knife to prevent scraping the cup.

6.1.5. After mixing, you will have a long time to work with the liquid solution, which on its own takes ~ 2-3 days to harden.
NOTE: The "base" is actually silicone oil, which is a long chain liquid polymer. The curing agent is a cross-linker that "welds" the long chain polymer base together.

6.1.6. Place the cup in the desiccator for 15 minutes to remove the bubbles. Ensure the red valve on the desiccator is open (parallel). Turn on the pump and set it at -20" Hg. Alternatively, you can turn on the pump and wait for the pressure to drop to 15mm Hg than turn the desiccator valve off (perpendicular) and turn the pump off.

6.1.7. After 15 minutes turn the pump off. Make sure the desiccator valve is open (parallel) and remove the red plug to release the pressure. Then you can remove the cup.
NOTE: Do not leave the pump running for an extended period of time (15-20 min is sufficient).

6.1.8. If bubbles remain, gently blow on the surface with compressed air.

6.1.9. Pour the requisite amount of mixed PDMS into the master mold and ensure the entire mold surface is covered. This can be done by eye or, more accurately, by weighing it out. Place the mold on the balance, and tare it, then pour in the PDMS.

6.1.9.1 To ensure the entire mold is covered and to minimize bubbles:

6.1.9.1.1 Hold the rim of the cup close to the wafer surface (~1/4”-1/2”).

6.1.9.1.2 Pour the PDMS near the edge of the mold away from the SU-8 channel structures. Pour in the direction of the channels (go with the grain).

6.1.9.1.3 Allow the PDMS to wet the entire exposed wafer and

channels.

NOTE: If spreading is too slow, you can tip and roll the master to accelerate the process and ensure total PDMS coverage.

6.1.10 Place the lid on the petri dish and place it in the desiccator for 15 min. Turn on the pump and set it at -20” Hg. Alternatively, you can turn on the pump and wait for the pressure to drop to 15mm Hg than turn the desiccator valve off (perpendicular) and turn the pump off.

6.1.11 After 15 minutes turn the pump off. Make sure the desiccator valve is open (parallel) and remove the red plug to release the pressure. Then you can remove the petri dish.
NOTE: If bubbles remain, gently blow on the surface with compressed air. Any remaining bubbles can be popped with a syringe tip or other sharp object.

6.1.12 Leave the petri dish on a flat, level surface for 5 minutes for the free surface to recover from bubble removal.

6.1.13 Then place the petri dish in a 60^{o}C oven for 1 hour. Make sure the oven shelf is level. If you need to place your master on the bottom shelf, make sure you use one of the cork spacers to avoid melting the petri dish.
NOTE: PDMS can be baked for longer/shorter than 1 hour giving a slightly firmer/softer elasticity.

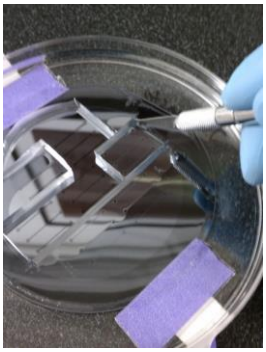
6.2. Removing PDMS Channels from the Mold

6.2.1. Remove the Petri dish from the oven and let it sit for 10-15 minutes to cool down.

6.2.2. Turn on the hotplate so it has time to warm up.

6.2.3. Using a razor blade cut around the inner perimeter of the SU-8 mold. Do not cut and remove the thick PDMS ring holding the master wafer to the petri dish.
NOTE: When cutting use minimal downward force, to avoid cracking the master.

6.2.4. Make a series of cuts and try to leave a ~5mm border of PDMS around each channel. This border will allow for a more secure bond to the glass substrate.



NOTE: Take care not to damage the SU-8 structures when cutting.

6.2.5. Remove any pieces of PDMS, which are not microchannels, from the mold. If needed, use tweezers to pry them out.

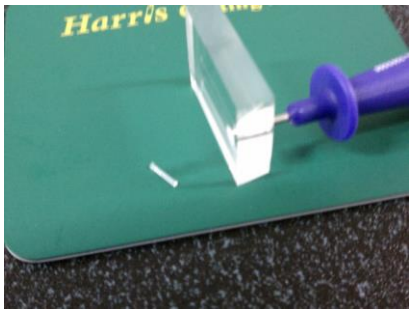
6.2.6. To avoid tearing, gently remove your PDMS channels from the mold by peeling them out slowly. Once removed, avoid touching or breathing on the channel (the molded surface) and minimize exposure to dust, as the channels are as small as a piece of dust.
NOTE: You can place a strip of Scotch tape over the channel to keep the surface clean and protected for storage or later use (they should be viable almost indefinitely).

6.2.7. Make sure the cutting mat is clean and dust free. Then place the PDMS microchannel channel side up on the cutting mat.

6.2.8. Holding a punch perpendicular to the PDMS slab, punch a hole in one of the circular wells of the channel. Having a light source, at a 10-30o angle, can help you find the wells.

NOTE: The punch size should be the same size as the O.D. of the experiment tubing (use 1.5 mm punches to for regular Tygon tubing). When punching the PDMS, compression of the material results in a well diameter that is smaller than the punch size. During channel assembly, this smaller well diameter is usually ideal for creating a sufficient seal to the tubing.

6.2.9. With the punch still in the PDMS, press the ejector on the opposite side of the punch to eject the PDMS plug. Make sure that the cut is complete and that plug is removed



from the well.

6.2.10. Remove the punch by gently twisting and pulling on the punch.

6.2.11. Repeat steps 6.2.6-6.2.8 for the remaining wells.

6.2.12. Cover **BOTH** the **channel side** and **top side** of the PDMS slab with Scotch tape to protect the channel and wells.

6.3. Bonding PDMS Channels to Glass

NOTE: It is best to use powder-free gloves to avoid leaving finger prints on the channels. Also, this process should be completed on a clean, dust free surface (e.g. the cutting mat).

6.3.1. Clean a glass slide by spraying it with 100% EtOH and wiping it with a kimwipe. Allow the slide to dry.

6.3.2. Right before bonding, dust the glass surface with compressed air.

6.3.3. <u>Non-Permanent Bond</u>

6.3.3.1. Remove Scotch tape from the channel side of the PDMS microchannel.
6.3.3.2. Align and stick the channel onto the glass substrate.

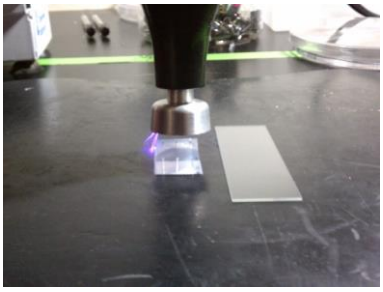
NOTE: A non-permanent bond between glass and PDMS is a result of van der Waals forces. This requires that both surfaces

be very clean. When using these channels, the pressure should be minimized (avoid excessive flow rates). Alternatively, the microchannel flows may be driven by negative pressure.

6.3.4. Permanent Bond

6.3.4.1. Remove Scotch tape from the channel side of the PDMS microchannel.

6.3.4.2. Place the sides to be bonded face-up on a non-conductive surface (e.g. rubber cutting mat). Ensure they are dust free.



6.3.4.3. Wave the high frequency coil ¼" – ½" above the surface of both the glass and PDMS for ~1 minute.

6.3.4.4. Lay the channel on the glass surface starting from one edge. Press down gently on the PDMS to ensure good contact.

NOTE: As the channel touches the glass, you should see the contact line propagate as the surfaces stick together. At this stage, inspect the device for air pockets and try to remove as many as possible.

6.3.4.5. If a misalignment occurs, peel them apart, briefly re-expose to plasma, and re-bond.

6.3.4.6. Immediately place the mated channel on a ~95^{o}C hotplate for up to 1 hour. Usually a permanent bond occurs within ~15 minutes if the plasma is sufficient.

NOTE: a permanent, covalent bond will form between the glass and PDMS within ~24 hours if left undisturbed. Alternatively, this process is accelerated dramatically by heating the channel to ~95^{o}C for up to 1 hour (5-10 min are usually sufficient at this temperature).

6.3.4.7. Remove from the hotplate and allow the channel to cool. Now the channel is ready for use or storage in a clean dry container.

Appendix A

References:

Xia and Whitesides, Ann. Rev. Mat. Sci. (1998)
Information on general soft lithography techniques

Haubert et al., Lab on a Chip (2006)
Details on permanent bonding