



Introduction:

In this session we will be demonstrating a simple microfluidics technique: selective dosing of cells in parallel laminar flows. While the field of microfluidics is rapidly advancing and complex operations have become possible, we will show how even simple devices that are inexpensive and relatively easy to fabricate can be useful tools. Each group will fabricate their own microfluidic channels, followed by a demonstration of the properties of laminar flow inside such channels. Finally, we will use the laminar flow of our channel to selectively treat cells, which would allow one to rapidly compare the response of dosed and control cells within one sample.

Fabrication of PDMS Microfluidic Channels

Sample Prep Procedure

Making the Master with Photolithography

Begin with a clean silicon wafer.

First you need to obtain a mask with patterns for the channels you wish to form. Typically these are drawn in a CAD program and printed on transparencies. For small channels with well-defined edges these types of masks should be professionally printed. Most recently, we've had masks printed by www.pageworks.com, which can do resolutions down to 10-15 microns.

Spin coat your Si wafer with photoresist to thickness of desired channel's height. The photoresist we use is SU-8 (a negative photoresist from MicroChem).

Expose and develop the photoresist according to manufacturer's instructions. For more details on SU-8, see http://www.microchem.com/products/pdf/SU8_2002-2025.pdf.

Place patterned silicon wafer in petri dish, or other flat-bottomed dish

Alternative for Masters: For large channels, crude but functional masters can be made from a variety of materials. Lengths of wire, thin strips of metal, or pieces of glass can be used as molds, or you can also use a thin layer of Scotch tape. Simply mat a layer of tape to an appropriate thickness on a petri dish (one layer of tape is ~100 microns) then use a razor blade to cut out the shape of the desired channel and peel away the excess. Masters of these sorts will not have as well-defined edges and are more fragile, but are good for quick testing of channel geometries, etc.

Preparing the PDMS

Polydimethylsiloxane (PDMS) is a soft polymer that is widely used to make cheap, disposable microfluidic devices. The PDMS we use is Sylgard 184 from Dow Corning, a two-part heat curable system that is mixed 10:1 with the included curing agent.





Prepare enough PDMS to fill your petri dish to desired level (~1 cm). **Stir vigorously until well mixed.**

Mixing introduces bubbles that must be degassed. This can be done in a centrifuge for small volumes or with a vacuum chamber for larger amounts. Place the PDMS/dish into the chamber, seal the chamber, and turn on the vacuum pump. Leave for about 30 min. Break seal slowly, turn off pump, and remove PDMS.

Slowly pour PDMS on top of the photolithographed master. If bubbles form near the channel during pouring, gently move them away from the channel with tweezers. You can also wait a few minutes for bubbles to rise to the surface and pop, or place the PDMS-filled master into the vacuum chamber and repeat degassing procedure, but only for 5-10 min.

Place in oven at 80C for 45 min – 1 hr to cure the PDMS.

Once cured, carefully cut around the channel with a sharp razor blade. Do this on a firm surface to be sure you don't crack the master. The photoresist is brittle and can be easily damaged by the blade, so be cautious.

Peel cured channel out of dish, and place in a new petri dish. It's best to keep the surface in which the channel is imprinted as clean as possible.

Before sealing the channel to a glass coverslip, we use sharpened hollow wire to make inlet and outlet holes. Wire size is based on the size of the tubing to be used. This step is easy to forget, but if forgotten can be done after sealing.

Sealing the Channel with a Glass Coverslip

If necessary, clean two coverslips and the PDMS channel with isopropanol, rinse with deionized water, and dry with a stream of nitrogen. Keep them free from oils and dust.

The PDMS surface can be chemically activated by exposing it to an oxygen plasma. This activation will allow us to bond the PDMS to a glass coverslip to seal off the channel.

Place PDMS **channel-side-up** on one cover slip, and lay this and the second cover slip in the plasma cleaner. Run the cleaner at 40 Watts for ~25 seconds.

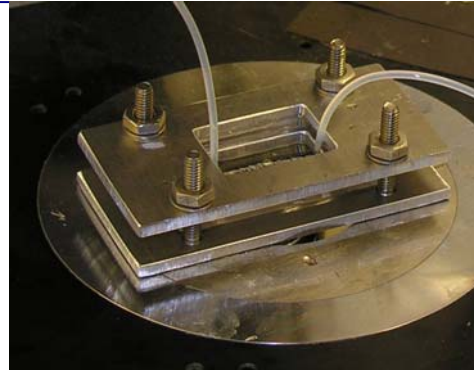
Remove them from the cleaner and immediately place the second cover slip on the channel side of the PDMS. Press gently and ensure no air pockets form near the channel. The cover slip should bond permanently to the PDMS (although if necessary the glass can be stripped away...usually piece by piece so use caution).

Slowly pry away the unbonded cover slip. Your microfluidics channel is now ready to use!





Alternative for sealing: If a certain application makes a permanent seal undesirable, a pressure fit can be a good alternative. PDMS wets well to glass; as long as high channel pressures are not required, a light pressure fit works well. At right is a picture of a frame we've built for this purpose.



Flowing Fluids

Cut off the sharp tip of a hypodermic needle and affix a few feet of Teflon tubing to the blunted needle. We use 21 gauge needles with Cole Parmer 06407-41 PTFE tubing.

Fill syringes with desired fluids, attach a syringe to each of the blunted needles, and place syringes in a syringe pump if desired (a gravity feed can be used as well). Insert the opposite end of the Teflon tubing into the input hole of the PDMS microchannel.

Repeat the preceding two steps for each input.

Affix an additional length of Teflon tubing to the output(s) of the microchannel. This can be fed into a waste vial. The channel is now ready for use with fluids.

Alternative for inputs/outputs: When placing input and output holes, keep in mind things like microscope objectives which may be in the way of tubing. If tubes emerging from the top of the PDMS are a hindrance, an alternative is to make two joining holes. Punch one hole halfway through the PDMS from the channel-side, and then punch another hole on the side of the PDMS which joins the first. Also, remember that inlets and outlets need not be vertical and can be angled. Disadvantages to these alternatives are they can make channels more fragile and prone to leaks.

Plating cells on the microfluidics channels. Mammalian cells will naturally adhere to glass. Adherent cells temporarily in suspension can be flowed into the channels, and will then settle and adhere. Cells that adhere quickly are the best choice for this experiment, since flow of media cannot resume until cell attachment has finished. PDMS itself can be made more attractive to cells by first flowing in fibronectin or collagen or polylysine as an adhesive substrate. Once cells have attached, a very slow flow of medium will keep the cells alive.

For more information, see the following:

Sia SK, Whitesides, GM., *Microfluidic Devices Fabricated in PDMS for Biological Studies*. *Electrophoresis* 24, 3563-3576, (2003).

