BE 167L - Bioengineering Laboratory

Lab 2: Laser Cutter, Fluorescent Microscopy, and Replica Molding

Replica Molding

Prelab reading

Read the article and watch video from JOVE "Microcontact Printing of Proteins for Cell Biology". PDF is posted on CCLE (note that a typo exists on page 3 of the print article, line 1, where hydrophilic should be hydrophobic). The full citation for the article is: Shen K., Qi J., Kam L.C. (2008). Microcontact Printing of Proteins for Cell Biology. JoVE. 22. Watch the primer video.

In this lab you will be creating several elastomeric stamps through the process of **replica molding** to be used in the next lab for **microcontact printing**. You should be conscious of getting practice with handling polydimethylsiloxane (PDMS), which is a very viscous liquid, and ways to measure its volume and transfer it reliably. Though your specific purpose in making these elastomers is for **microcontact printing**, this technique is also useful for applications in rapid prototyping and microfluidics. These replica molds don't have to be used as stamps.

Polydimethylsiloxane (PDMS) is a thermosetting polymer that is commonly used for replica molding. Any elastic polymer could be used for replica molding. PDMS has many desirable characteristics:

- It is moderately stiff (shear mod.= 0.25 MPa, Young's = 0.5 MPa)
- It conforms to a surface with atomic level contact (good seals, microfluidic devices)
- It is non-toxic and commercially available ($80/kg)
- It is optically transparent to 300 nm
- It is hydrophobic, but can be made hydrophilic with oxygen plasma
- It can adhere and seal reversibly, or irreversibly after oxidation, to many different surfaces

You will be using an acrylic mold in a polystyrene petri dish. Many times you will see replica molds made on silicon wafers with patterns created by **photolithography**. Because of the infrastructure involved in photolithography on these silicon wafers, you will not make molds this way in the teaching lab. If you are interested in this process, you may read this article from Nature Protocols: *Soft lithography for micro- and nanoscale patterning, Qin et al., vol. 5, pg 491 - 502 (2010)*. There are several labs at UCLA which routinely use this process, and a clean room facility which you may end up using in the future if you join one of those labs.

Preparation

Reagents

- Sylgard 184 base
- Sylgard 184 curing agent

Supplies

- Plastic transfer pipettes
- Weigh boats
- Acrylic stencil molds in small petri dishes
- 1 large (100 cm) plastic petri dish
- 50 mL conical tube

Equipment

- Oven at 60°C

- Vacuum dessicator

Safety

Wear your usual protective equipment. Take care when handling the Sylgard reagents. They are very oily and difficult to remove. You may need to change your gloves frequently to keep from spreading this reagent to your bench space, equipment, and clothes. Keep some paper towels nearby to help contain any spills or accidents.

Procedure

Acrylic stencil molds have been prepared already. Features were etched in the hard acrylic with a laser cutter. Please perform handling of PDMS solutions inside the fume hood. All the necessary supplies for this lab will be located in the fume hood.

1. PDMS is oily and may be difficult to remove from surfaces. If gloves become contaminated with PDMS, replace them before touching anything else, especially the balance and the vacuum chamber.
2. The recommended base to curing agent ratio for Sylgard 184 (PDMS) is 10 to 1 by weight. Estimate the total volume that will occupy the acrylic mold.
3. Weigh your components in a 50 mL conical tube using the scale located in the fume hood.
4. Stir to mix the two components with the transfer pipette. After mixing well pour the solution into the mold provided.
5. Degas the PDMS. Place the molds in the large plastic petri dish with your initials on it. You may share a vacuum dessicator with other teams; wait until your vacuum dessicator is full before starting. Place the petri dish in the vacuum chamber. (Check with the TA before continuing) Close the chamber, ensuring the o-ring is properly situated. Connect the chamber to the vacuum line coming from the bench top. Make sure the valve on the vacuum chamber is open, and then open the vacuum line. Air within the PDMS will leave via bubbles. Be careful not to tip the chamber or you will spill your PDMS. This process should take approximately 30 minutes. Record the time you started the vacuum and how long it takes to remove the bubbles.
6. Once all the bubbles have disappeared, close the valve on the vacuum chamber, close the vacuum line, remove the vacuum line, then reopen the vacuum chamber *slowly*. Be careful not to tip the chamber again, or you may spill your PDMS since it is not cured yet. Remove the lid of the vacuum chamber and place the petri dish of molds in the oven, set to 60°C. They will be removed by your TA. Make sure your initials are on your petri dishes.

Fluorescence Microscopy

Procedure

1. Obtain and clean a glass slide and cover slip with 70% ethanol and a kimwipe.
2. Dispense 10 µL of the fluorescent bead suspension (provided by the TA) onto the glass slide.
3. Place a cover slip on top of the drop. Bring the slide to a fluorescent microscope for examination.

Safety

Wear gloves to protect yourself from any substances you are handling and to keep the equipment clean, but take your gloves off to use the computer keyboard and mouse.

Using the AMG EVOS Microscope

Your TA will demonstrate how to use the microscope in class. Be sure to follow along and write down your own procedure, and ask the TA to clarify as necessary. Take pictures of the fluorescent beads so that you can measure their size for your lab reports.