Systems *Bioengineering* Laboratory

Microcontact Printing of Surface Proteins

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Materials

- PDMS, Polydimethylsilaxane (Slygard 184, Fisher, Cat: NC9644388)
- Glass goverslips, such as 22x22-1 (Fisher, Cat: 12-548-B)
- Style 2-A Forceps (EMS, Cat: 72919-2A)
- Tween-20 (Sigma, Cat: P1379)
- Pluronic Solution (0.2% in diH_2O diluted from 1% stock; prepared ahead of time)
  - Pluronic F-127 (Sigma, Cat: P2443)
- Liquid Silanization Solution (5% silane in benzene)
  - 1,2 dichlorobenzene, anhydrous (Sigma, Cat: 240664)
  - Dicholorodimethylsilane (Sigma, Cat: 440272)
- Acetone (Fisher, Cat: A18-1)

Methods

1. Pour PDMS on SU-8 patterned master wafer; aluminum foil the bottom of the master for easy removal.

   1.1. Use 1:10 base-curing agent combination; 10-15 mL of total volume for 90 mm dishes, or 20-30 mL for 150 mm dishes work best for this application

2. Ink the stamps:

   2.1. Remove the elastomeric negative from the master and cut out the desired patterns from the whole stamp. For easy stamping, keep 0.5-1 cm pieces (0.25-1 cm^2). *Optional:* Place the desired stamps on a plain glass microscope slide and plasma-clean for ~60 seconds just before use. This step is especially useful for stamping poly-L-lysine.

   2.2. Create a moisture-chamber by placing a 4''x4'' Parafilm at the center of a 150mm tissue culture dish and adding pieces of wet KimWipes at the edges.

   2.3. Add desired number of protein solution droplets (~40 µL for a 1 cm^2 stamp) on the Parafilm and place the stamps upside-down into the droplets paying close attention to induce complete contact with the protein solution. Incubate at room temperature for 1 hour.

   Note: If fluorescently labeled, keep in dark.

3. Prepare silanized glass coverslips:

   3.1. *Optional:* Immediately prior to stamping, plasma-clean the glass coverslips (that are already pre-cleaned with detergent and rinsed with diH_2O and Ni dried) for ~60 seconds. This step is highly recommended for even and consistent silanization of surface.

   3.2. Place small amount of silanization solution into a glass dish (silane reacts with everything) under a chemical hood.

   Note: Both benzene and silane are highly volatile and toxic; ensure proper safety.

   3.3. Add the coverslip into solution, swirl to ensure even coating, and incubate 20 seconds.

   3.4. Rapidly move the coverslip into acetone and wash for 10 seconds.

   3.5. Wash in running ddH_2O for 30 seconds (as an option, use two consecutive diH_2O baths prior to running water wash for complete removal of acetone and residual silane).

3.6. Blow dry coverslip with nitrogen.

4. Stamp the desired protein:

4.1. Rinse the pattern-to-be-stamped with PBS, PBS-Tween, PBS and ddH_2O (by dunking rapidly x30-50)

4.2. Blow dry stamp with nitrogen without rewetting the surface.

4.3. Put the glass coverslip on a large glass slide or culture dish; carefully grab the stamp and orient it at a negative angle (such that when it's upside down, forceps point down as well).

4.4. Slowly place the stamp face down on the coverslip in one smooth motion. Use forceps to gently reinforce complete contact -- do not use excessive force since that may induce bending and over-contact between patterns.

4.5. Gently place 12-50 g of weight (per cm^2) on top of the stamp to establish firm contact between the PDMS stamp and the coverslip. A steel hexnut (3-4 g) is usually adequate for a standard 0.25 cm^2 stamp. If needed (i.e. poor transfer, pure edge contact, etc) use a steel bolt or thumbscrew (10-15 g).

4.6. Wait for 30-120 seconds for adsorption of proteins on the coverslip. Transfer is likely to be an instant process; be consistent.

4.7. Carefully remove the weight. *Optional:* Remove the stamp without any lateral motion; any motion may cause a smeared pattern or dislocation and destruction of transferred protein.

5. Coat the surface with Pluronic:

5.1. Slowly add the Pluronic solution (without moving the stamp, if it is still on there) making sure it covers the entire coverslip. Incubate at room temperature for 1 hour. Remove the stamp after incubation.

5.2. Carefully start washing with ddH_2O while aspirating Pluronic; ensure that the surface is never exposed to air during this process. Use adequate water to remove all Pluronic; this may take 50-100 mL per coverslip.