Lipodrop 2.0 microchip fabrication

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AIM:

To create microfluidic chips for liposome synthesis.

REAGENTS USED:

Table 1. List of reagents used in the experiment

Name of reagent
SU-8 3010
Developer
Isopropanol
Elastomer
Curing agent

Note: *The ratio between elastomer and curing agent should be 10:1*

EXPERIMENT DESCRIPTION:

This protocol is meant to go through the steps of photolithography to create a microfluidic device ***lipodrop 2.0*** for the production of homogenous liposomes. The simplified scheme of the whole process is illustrated in **Fig. 2.**

Figure 1. PDMS microfluidic device lipodrop 2.0

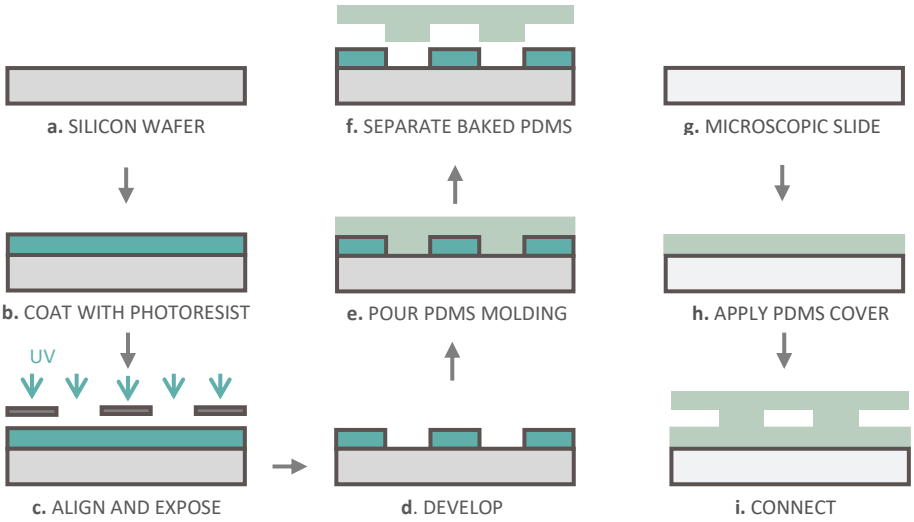

Fig. 2 *Simplified scheme for microfluidic device preparation.* ***a-b*** *Silicon wafer is cleaned and spin-coated with photoresist;* ***c*** *photomask is aligned on the sample and exposed to UV light.* ***d*** *sample is submerged to developer – only the sections exposed to the UV light remain on the wafer;* ***e*** *PDMS is poured onto the master to create PDMS mold and left for a bake in the oven;* ***f*** *the mold is then separated and prepared further by cleaning and punching inlets and outlets;* ***e-f*** *microscopic slide is prepared by applying a thin layer of PDMS on top;* ***i*** *PDMS mold and PDMS covered microscopic slide are plasma treated and connected to each other to produce a final microfluidic chip.*

EXPERIMENT PROTOCOL:

1. **Prior preparations**

   Gas supply is enabled by turning the valve of the oxygen container. UV lamp power supply is powered on, and the Hg lamp is ignited by holding down the “Start” switch. Vacuum pump on the lower right of the outer fume hood is turned on. A red lever in the fume hood should be pointing to the left for the coating process, while it should be pointing right while debubbling of the PDMS. Heating pads are set to 95 ℃ and 65 ℃. A fresh batch of developer is poured into a glass plate (while the used one is removed to appropriate waste container) and kept closed when not used. A few mL of the developer is sucked into the syringe and left in the fume hood for later use.

2. **Silicon wafer preparation**

   A silicon wafer is cleaned upon clean nitrogen gas stream in the fume hood. If the wafer has visible dirt, a wash with isopropanol is recommended. Continually, the wafer is etched by plasma to remove all of the organic material:

   - A plasma chamber is turned on (red button on the lower-right on the front side)
   - Ventilation is turned on for around 10-15 seconds; a lid is then removed
   - Clean wafer is placed in the middle of the chamber
   - Pump is turned on and the lid is pressed to the right side until firmly sucked
   - Pump and ventilation are on for 10-15 seconds

- Ventilation is then turned off while pump is on for another 30s
- Gas is turned on and tuned to 10 with a black screw below the buttons
- After 20s, the gas value is readjusted to 2.5 (time value should be kept at 25s)
- Generator is turned on. Generator button turns off automatically after the process has ended
- Gas is turned off and ventilation is turned on (pump should still be on)
- After 10s pump is turned off, leaving only ventilation on
- After 30s the chamber is opened and the wafer is removed
- The lid is closed immediately as described previously
- All of the buttons are turned off

If needed, the wafer is additionally cleaned under a clean stream of nitrogen gas in the fume hood.

3. Spin Coating Operating Procedures

1. Power up spin coater by pressing in the switch (pin) on the back right of the spin coater
2. Power on vacuum pump (the button is on the lower right of the outer fume hood)
3. Data sheet for the SU-8 3010 can be found here: http://microchem.com/pdf/SU-8%203000%20Data%20Sheet.pdf Photoresist SU-8 3010 should be stored in a dark area with a cap on
4. Spin coating procedures for a Si wafer are stored under Dynamic Process:
    - (1) Spin at 500 rpm for 5-10 s with acceleration of 100 rpm/s
    - (2) Spin at 3000 rpm for 30 s with acceleration of 300 rpm/s
    - (3) Spin at 1000 rpm for 60 s with acceleration of 200 rpm/s *(this step is for developer dispension – see step 3.9 below)*
    - (4) Spin with deceleration of 200 rpm/s until stopped
5. When the program is set, **press Run** button
6. Place the wafer on the spin coating chuck and align to the center of the chuck
7. **Press Vacuum** to hold the device to the chuck
8. Pour a reasonable amount (~5mL – it is dependent on surface area) of photoresist on the wafer and close the lid and immediately start the spin coating process
9. During the 3rd cycle a syringe is extended through the hole on the lid and the developer is uniformly dispensed on the edge of the wafer (up to 2 mL).
   Note: *Cleaning should be performed for 40 seconds and after that 20 seconds should be left to get rid of the developer from the wafer.*
10. After spin coating, open the lid and **press Vacuum** button. Carefully lift the device off the chuck and close the lid

4. Soft Bake

The wafer is placed on the 95 ℃ heating pad for 2-3 minutes (4 – 10 µm) or 5-10 minutes (8 - 15 µm). After the soft bake, a heating pad is turned off prior to preparation for step 7.

5. Exposure

While the wafer is baking, an exposure frame is prepared: the black glass is put on the plastic frame. After baking, the wafer is then carefully placed in the middle of the frame. A mask is centered on the wafer. A special quartz glass transparent to UV light is cleaned under a stream of nitrogen gas and carefully placed on top of the wafer. The exposure frame with the wafer is aligned under the UV lamp. A mouse pad is then used to cover

the lamp. An exposure time is set to 20s. **Press Expose** button. A loud clunking noise indicates a successful initiation of the process.

6. Post Exposure Bake

- Directly after exposure a wafer is placed on the 65 ℃ heating pad for 1 minute.
- It is then placed on the 95 ℃ heating pad for 1-2 minutes (4 – 10 µm) or 2-4 minutes (8 - 15 µm)

Note: *After 1 minute of PEB at 95 ℃, an image of the mask should be visible in the SU-8 3010 photoresist coating. No visible latent image during or after PEB means that there was insufficient exposure, temperature or both.*

6. Develop, Rinse and Dry

The wafer is immersed into a fresh developer that was poured into the glass plate for 1 – 3 minutes (4 – 10 µm) or 4 – 6 minutes (8 – 15 µm). An orbital shaker should be turned on during the process. Right after the immersion, the wafer is cleaned for 10 s under a stream of developer (red plastic container) into the appropriate waste container. A stream of isopropanol is used to clean the wafer for additional 10 s. A stream of clean nitrogen gas is used in the fume hood to dry the wafer.

7. Hard Bake

A wafer is placed on a room temperature heating pad. Temperature is gradually increased until it has reached 150-180 ℃ and left for 15-60 minutes. After the bake, the wafer is placed into a plastic petri dish (it should already contain written information on the bottom).

8. Preparation of PDMS

- A Desiccator oven is turned on and heated up to 70 ℃
- 30 g of elastomer is weighed into a clean plastic cup
- 3 g of curing agent is weighed to the container with the elastomer

Note: *When making a new device 30 g elastomer and 3 g of curing agent is used while 10 g and 1 g are used while refilling.*

- All of the components are thoroughly mixed with a plastic fork until a cloudy consistency is produced
- PDMS is separated equally into two plastic cups (one cup should contain no more than 60 g of PDMS)
- The PDMS is then placed in the Desiccator oven until most of the bubbles are eliminated.

Note: *A ratio of elastomer and curing agent should always be 10:1 (it is allowed to have an excess of elastomer up to a ratio of 15:1).*

9. Application of PDMS to the wafer

Up to 30 g of PDMS is poured onto the wafer a bit like how you would apply frosting to a cake. It is then placed in the Desiccator oven for additional bubble removal (5 minutes). If air is observed under the wafer, it can be pressed out with a fork. The last remaining bubbles are destroyed with a pumping device.

Note: *TRY NOT to have any bubbles in the PDMS within the mold because the bubbles will solidify in the baked sample.*

10. Baking of PDMS

The Petri dish is placed into the oven overnight.

11. Preparation of the glass substrates

Glass slides are cleaned with clean-room wipes and isopropanol. A thin layer of PDMS is applied on the glass substrates by methods described previously (Steps: 8-1).

Note: *The glass slides are placed on a wafer that are covered with aluminum foil layer placed underneath the wafers (the foil prevents the overflow of PDMS). The baking time is at least 2 hours.*

12. Post preparation of the mold

- A microchip is cut out according to the outer lines of the pattern with a sharp scalpel. An air bubble that is observed along the cutting line indicates that the scalpel reaches the substrate
- An already cut mold is carefully removed
- The upper part of the mold (the one without the channels) is taped with a tape (tape strips should overlap)
- The mold is taped on the cleanroom wipe with the channels facing upwards
- The octopi's lamps are regulated in a way where the patterns of the device are visible
- A piece of tape is stuck to the regular table lamp in order to clean up the pincher regularly (especially the post junction channels)
- Green biopsy punchers are used to punch holes of the indicated inlets/outlets. The post junction channels (the filterless ones) are pinched first.
  Note*: a puncher should be placed vertically during the process.*
- The tape and punched PDMS are removed with tweezers.
- The mold is cut to a rectangle shape with a guillotine.
- The mold is submerged in a glass container with isopropanol and placed in the ultrasonic bath up to 30 seconds.
- A stream of clean nitrogen gas is used to dry the mold in the fume hood.
- The mold is put to the oven until plasma chamber is ready *(See step 14)*.

13. Post preparation of glass slides

The glass slides are cut out from the plate with PDMS. They are then cleaned with isopropanol and dried under a stream of nitrogen gas.

14. Microchip preparation

- The glass slides (PDMS facing up) and the molds (channels facing up) are plasma etched (Step 2)
- The molds are carefully placed on top of PDMS-coated glass slides. The channels should be facing down. ***Note:*** *the mold should touch the slide center first. The mold should stick to the glass without leaving any bubbles. Any remaining bubbles are removed by gently pressing it with the whole finger*
- The top of the microchip is covered with tape
- The microchip is then placed on the heating pad heated to 120 ℃ for 30 minutes.

ADDITIONAL OBSERVATIONS AND IDEAS:

- Make sure that the cooked PDMS is always facing the appropriate direction at all times.
- When cutting out the PDMS, be aware about the applied pressure especially around the edges to avoid breaking the wafer.