

Protocol for culturing cells on micro-pattern (TC Qian)

1. Design pattern by AutoCAD, we can order printing service from

<http://www.thinmetalparts.com/fineline/>

a) Add more details

2. Make patterns on silicon wafer by photolithography

1). To obtain maximum process reliability, substrates (maker, catalog number, link, got from Tong Chen) should be clean and dry prior to applying the SU-8 2000 resist.

Start with a solvent cleaning, or a rinse with dilute acid, followed by a DI water rinse.

Where applicable, substrates should be subjected to a piranha etch / clean (H₂SO₄ &

H₂O₂=3:1 (V/V), or (Nong² Liu² Shuan¹ and NaOH, from the clean room

concentration?). To dehydrate the surface, bake at 200°C for 5 minutes on a contact

hot plate. Mark the steps that can be trained in the clean room.

2). Choose the spinning speed according to the desired thickness. Program the spinning coating machine. (For example by using SU-8 2015 to get 20 μm thickness, Ramp to 500 rpm at 100 rpm/second acceleration. This will take 5 seconds. Spin Cycle: Ramp to final spin speed at an acceleration of 300 rpm/second and hold for a total of 30 seconds.)

3). Place silicon wafer on the chuck to test the position until the wafer stay steady on the chuck.

Dispense approximately 1ml of SU-8 2000 per inch of substrate diameter.

4). Soft bake: follow the protocol provided by the SU-8 2000 according to the desired thickness ((For example by using SU-8 2015 to get 20 μm thickness, 4min at 95 °C)

- 5). Exposure : Exposure time should be calculate according to the desired thickness
(For example by using SU-8 2015 to get 20 μm thickness, $150\text{mJ}/\text{cm}^2$)
- 6). Post expose bake: follow the protocol provided by the SU-8 2000 according to the desired thickness (For example by using SU-8 2015 to get 20 μm thickness, 4min at $95\text{ }^\circ\text{C}$)
- 7). Developing: Use SU-8 developer, developing time should be calculated according to the desired thickness.(For example by using SU-8 2015 to get 20 μm thickness, develop the wafer for 4min).

3. Preparation of the micro patterns:

PDMS molds were achieved by remolding the silicon masters developed by photolithography. Subsequently PDMS molds were treated with oxygen plasma at the power of 100W for 30s for the purpose of hydrophilic modification of the surface, followed by rinsing the molds in a comb polymer solution (60 mg/ml in 8:2 (vol./vol.) mixture of ethanol and deionized water) for 10 seconds, finally the molds were spun cast at the speed of 3000rpm for 15 s, and the micro patterns were gotten by micro contact printing (μCP) from the PDMS molds.

4. Pass cells onto patterns

Before pass cells, the dishes with micro-patterns should be exposed to UV light for 30min, then coat Fn on the pattern, incubate the Fn coated dishes for around 2-4h, then rinse the dishes with PBS once, after that, the dishes with patterns are ready for

passing cells.