Supplementary Material (ESI) for Lab on a Chip

Supplementary protocol: 2D cell culture in a poly(dimethylsiloxane) (PDMS) microfluidic perfusion culture system

This example protocol illustrates a typical 2D microfluidic perfusion culture experiment. We assume the experiment involves non-recirculating flow in a device comprised of a 50-μm-high PDMS microfluidic network; glass or tissue-culture polystyrene cell culture substrate; and HPLC tubing and valves (made of Teflon® and PEEK). The cell suspension is loaded by hand. During culture the device is placed inside a standard tissue culture incubator, connected by tubing to a syringe pump drive outside of the incubator.

Although many of the detailed steps are specific to these assumptions, this protocol is intended to give a general idea of what types of steps are involved during a typical experiment.

[A] Fabrication of PDMS microfluidic network

1. Fabrication of silicon masters for replica molding
    a. Microfluidic networks are designed with software such as AutoCAD (Autodesk, USA) or L-Edit v10.20 (Tanner Research, USA).
    b. Send out design to commercial photo-plotting companies *e.g.*, Fineline Imaging, USA to obtain transparency photomasks
    c. Spin coat a layer of SU-8 photoresist (SU-8 2035, MicroChem Corp. USA) onto a 4" silicon wafer.
        i. Dispense 4 ml of photoresist onto the silicon wafer.
        ii. Spin at 500 rpm for 5-10 seconds with acceleration of 100 rpm/second.
        iii. Spin at 2000 rpm for 30 seconds with acceleration of 300 rpm/second.
    d. Pre-exposure bake: 65^{o}C for 3 minutes followed by 95^{o}C for 9 minutes.
    e. Photolithography to transfer microfluidic network pattern on photomask onto the photoresist using a UV lamp below 350 nm.
    f. Post-exposure bake: 65^{o}C for 2 minutes followed by 95^{o}C for 7 minutes.
    g. Develop with SU-8 developer (MicroChem Corp., USA) for 5-7 minutes. Rinse with fresh developer, followed by Isopropyl alcohol (IPA) and air dry.

For feature sizes larger than 50 μm, low-cost molds are commercially available e.g. Fineline Prototyping, USA.

2. Replica molding with PDMS
    a. Prepare PDMS polymer (Sylgard 184, Dow Corning, USA) by mixing 10 parts PDMS prepolymer with 1 part curing agent (measure by weight).
    b. Place into vacuum chamber to degas PDMS polymer for 30 minutes.

c. Pour PDMS polymer over silicon mold and place into 60^{o}C oven to cure for 2 hours.
d. Remove PDMS microfluidic network from silicon mold. Rinsing with IPA helps in lifting the PDMS slab from the mold.

3. Sealing of PDMS microfluidic network to glass
    a. Clean glass microscope slides or coverslips by soaking with 5M NaOH for 1 hour. Rinse with distilled water and air dry.
    b. Clean PDMS microfluidic network with detergent and distilled water. Blow dry to remove remaining dust particles.
    c. Expose both glass slide and PDMS slab to oxygen plasma for 1 minute (at 125 W, 13.5 MHz, 50 sccm, and 40 millitorr) and place the PDMS microfluidic network onto the glass slide to seal it.

Or

4. Sealing of PDMS microfluidic network to tissue culture polystyrene
    a. Clean PDMS microfluidic network with detergent and distilled water. Blow dry to remove remaining dust particles.
    b. Coat tissue culture polystyrene plate with 10% - 100% (v/v) of Sylgard PrimeCoat (Dow Corning, USA) diluted in heptane. Incubate at room temperature for 1 hour.
    c. Expose both coated tissue culture polystyrene plate and PDMS slab to oxygen plasma for 1 minute (at 125 W, 13.5 MHz, 50 sccm, and 40 millitorr) and place the PDMS microfluidic network onto the tissue culture polystyrene plate to seal it.

[B] Operation of the PDMS microfluidic perfusion system for 2D cell culture

Preparation Day:

1) Equilibrate culture media to correct pH by incubating a dish of culture media in a standard CO_2 incubator for > 4 hours.
2) Sterilize the system
    a. Place PDMS microfluidic device, associated tubing and off-chip valves inside an autoclaveable packet (ChexAll II Pouch, Propper #024014). Autoclave (121°C for 8 minutes wet, 15 minutes dry) Next place autoclaved packet in a 60°C oven for >2 hours to dry PDMS.
    b. Sterilize polystyrene tissue culture base by exposure to UV light for 1 hour in biosafety cabinet.
3) Assemble the system in a sterile environment
    a. Assemble a leak-tight microfluidic system in a sterile environment (for example, inside a standard biosafety cabinet).
    b. Using 0.2 micron syringe filters (Acrodisc filter, Pall #4602) at syringe inputs where cells will not be loaded (for example, at culture-media syringe inputs) helps maintain a sterile environment.
    c. Fill a syringe (5-10 mL) with 0.1% gelatin or other ECM-type material and attach to the microfluidic system. Flush system with gelatin solution.
4) Debubble the system

a. Debubble the system by perfusing the system with the gelatin solution at a pressure >5 psi for >2 hours. To achieve high pressures at low flow rates, use output tubing with a high fluidic resistance. *Pressure=(fluidic resistance)× (flow rate).*
b. After all bubbles are eliminated, stop perfusion and allow the gelatin to incubate in the device at room temperature for 1 hour.

5) Begin perfusing the system with equilibrated culture media to rinse and equilibrate device.
    a. In a sterile environment, fill a syringe (5-10 mL) with equilibrated culture media and connect to microfluidic system, using droplet merging when attaching the syringe.
    b. Perfuse system with culture media overnight. (Can be performed outside the hood as long as the system is leak-tight and perfusion is continuous, so that bacteria may not enter through the system outputs.)
6) Prepare static control: Meanwhile, prepare a conventional tissue-culture polystyrene dish static control by incubating the surface with the same 0.1% gelatin used in the device for 1 hour. After one hour, aspirate gelatin solution and pipet culture media into dish. Incubate overnight.

Day 0: Load cells

1) Prepare the microfluidic system
    a. Bring the microfluidic system into a sterile environment
    b. Where possible, backflow media out of the inlet where the cells will be loaded. This prevents air bubbles from being loaded into the device while cells are loaded.
2) Load cells
    a. Trypsinize, count, and suspend cells at a known density in equilibrated media. Quickly (within a few minutes) load cell suspension into a syringe, taking care to avoid loading air bubbles into the syringe. Orient the syringe handle-up and wait ~20 seconds to allow any air bubbles to rise to the handle portion of the syringe. Then attach the syringe containing the cell suspension to the microfluidic system (using droplet merging to avoid air bubbles) and load the cells by hand into the device.
3) Meanwhile plate cells in the standard tissue-culture polystyrene dish as a static control. Ensure that the seeding density is the same as in the microfluidic device. (This will involve making a more dilute cell suspension.)
4) Allow cells to attach under static conditions
    a. After the cell suspension is loaded, close off all inputs and outputs to the device and place the entire system into a standard tissue-culture incubator. Allow cells to attach under static conditions for >4 hours (depends on cell type and media).
5) Start perfusion
    a. Using a syringe pump outside the incubator, perfused equilibrated media through flexible tubing that enters the incubator using an air-filter port. Attach the tubing to the microfluidic system using standard HPLC parts.

Day >1:

1) At later time points, the microfluidic device may be disconnected from the culture media feed and removed from the incubator for imaging. Sterility is maintained by placing a syringe filter at the input to the microfluidic system.