Video Article

Patterning of Embryonic Stem Cells Using the Bio Flip Chip

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Abstract

Cell-cell interactions consisting of diffusible signaling and cell-cell contact (juxtacrine signaling) are important in numerous biological processes such as tumor growth, stem cell differentiation, and stem cell self-renewal. A number of methods currently exist to modulate cell signaling in vitro. One method of modulating the total amount of diffusible signaling is to vary the cell seeding density during culture. Due to the random nature of cell seeding, this results in considerable variation in the actual cell-cell spacing and amount of cell-cell contact, and cannot prescribe the local environment. A more specific approach for modulating cell signaling is to use molecular inhibitors or genetic approaches to knock down specific signaling proteins, but both of these methods are best suited to manipulating small numbers of molecules. Here, we demonstrate a new approach to modulating cell-cell signaling that modulates the local environment of a cluster of cells by placing different numbers of cells at desired locations on a substrate. This method provides a complementary way to control the local diffusible and juxtacrine signaling between cells. Our method makes use of the Bio Flip Chip (BFC), a microfabricated silicone chip containing hundreds-to-thousands of microwells, each sized to hold either a single cell or small numbers of cells. We load the chip with cells simply by pipetting them onto the array of wells and washing unloaded cells off the array. The chip is then flipped onto a substrate, whereby the cells fall out of the wells and onto the substrate, maintaining their patterning. After the cells have attached, the chip can be removed (or left on). This approach to cell patterning is unique in that it: 1) doesn't alter the chemistry of the substrate, thus allowing cells to proliferate and migrate; 2) allows patterning onto any substrate, including tissue-culture polystyrene, glass, matrigel, and even feeder cell layers; and 3) is compatible with traditional microcontact printing, allowing the creation of extracellular matrix islands with cells placed inside those islands. In this video, we demonstrate the patterning of mouse embryonic stem cells onto tissue-culture polystyrene using the BFC.

Video Link

The video component of this article can be found at http://www.jove.com/video/318/

Protocol

Making a BFC

BFCs are made by molding polydimethylsiloxane (PDMS) over a 4" Si master wafer.

Making a master wafer

1. Start by dehydrating the Si wafers for 30 min at 130°C.
2. Place the wafer on a spin-coater, pour SU8-2050 (Microchem) onto the wafer, ramp from 300 rpm/s to 3000-4000 rpm, and spin for 30 s to yield feature heights of 40-30 mm, respectively.
3. After spinning, place the wafer on a 65°C hotplate, immediately ramp up the temperature to 95°C for 5-6 min, and then ramp it down to 65°C.
4. Expose the wafers to a UV dose of 200 mJ/cm^2 on a contact aligner using a dark-field mask.
5. Place the wafers on a 65°C hotplate, immediately ramp up the temperature to 95°C for 4-5 min, and then ramp it down to 65°C.
6. Develop the wafers in PM acetate and Isopropanol.
7. Silanize the wafers for 30 min using hexamethyldisiloxane (Shin-Etsu MicroSi), or (Tridecafluoro-1,1,2,2-tetrahydrooctyl)-1-trichlorosilane (t2492-KG, UCT Specialities, Bristol, PA), to prevent PDMS from adhering to the Si master wafer.

Molding chips

1. Mix PDMS (Dow Corning, Sylgard 184) in a 10:1 ratio, pour it over the master Si wafer (~15 g per wafer), and let it cure overnight at room temperature.
2. Peel the cured PDMS off the Si wafer and cut out each chip using a razor blade.
3. Bond each chip to a 1x1 cm cut microscope slide for easy handling.

Preparing the BFC for use

1. Soak the BFC overnight in PBS in order to prevent absorption of media into the PDMS during the experiment.
2. Dry the chips with a Kimwipe (Kimtech Science).
3. Put a drop of 7.5% Bovine Serum Albumin (BSA) (~200 mL) on the patterned surface of the BFC, and then scrape it with a pipette tip to disperse the BSA and remove any bubbles from the wells.
4. Leave the BSA on the BFC surface for at least 0.5 hours. Ideally agitate the BSA every 15 min to prevent crusting.
5. To fit the BFC inside a 35x10 mm TCPS dish (Falcon, 35-3001), cut the rims of a TCPS dish half way down so that the ¾" binder clips (Office Depot) will fit over the dish rim to clamp the chip and dish together.
6. Cut a spacer gasket (frame-shaped with 20´20 mm outer edge, 15´15 mm inner edge, and 250-500 mm thickness) from a PDMS sheet (Silicone Specialty Products) or any other silicone, and apply it to the dish using tweezers.

Patterning cells with a BFC

1. Sterilize the dish, gasket, BSA-coated chip, and 2 binder clips under UV light for 1 minute.
2. Aspirate the BSA and rinse the BFC twice with 200 mL PBS.
3. Add gelatin to the cut TCPS dish if required for your cell-line. Otherwise, wet the TCPS surface with some media before applying it to the chip (see below). This prevents bubbles from forming in the chamber.
4. After aspirating the PBS, pipette 200 mL of cell solution (~1×10^6 cells/mL) onto the BFC surface. Let the cells settle for 5-10 min.
5. Tilt the BFC to one corner at a ~15° angle and slowly pipette the cell solution off with a 200 mL pipette. Then, place the BFC flat and add 100 mL of PBS or blank media to the opposite BFC corner. Rinse the BFC in this manner an additional 2-4 times, as necessary, to clear all cells from the inter-well regions.
6. Pipette 200 mL of media onto the chip surface. Aspirate gelatin/media from the TCPS. Then invert the pre-wetted dish and slowly push the BFC up, into the dish.
7. Apply a binder clip each, to two sides of the chamber to seal it. Remove the metal prongs from the binder clips so that dish remains level when flipped over.
8. Finally, quickly flip the setup over while avoiding any unnecessary movement, and place it in the incubator.

Discussion

Although the protocol and video shown here describe using the BFC [1] to pattern mouse embryonic stem cells (mESCs), BFCs can be used to pattern practically any cell type of interest. The only change one may need to make is to alter the size of the microwells. In our experience, to use the BFC to pattern single cells of another cell type, a microwell diameter and height equal to 10 microns greater than the unattached cell diameter works best.

BFCs can also be used to pattern cells onto other substrates, including other cells, without significant changes in the protocol. Patterning onto an existing cell layer is one advantage of the BFC approach over traditional cell patterning approaches since certain celltypes, including mESCs and human ESCs, require support or feeder cells to maintain their proper phenotype in culture.

One application of the BFC that we are pursuing is to use the BFC to investigate signaling between mouse embryonic stem cells. By seeding single cells in square lattices with different grid spacings, we modulate the rate at which signaling molecules are exchanged by cells. By making larger wells (with a diameter of 40 microns), we can also collect larger numbers (~2-10) cells at a given location, locally altering cell density. In addition, we can place a number of small wells in close proximity to each other, resulting in a substrate pattern where local cell density is high by cells are not in contact. In this fashion, we can independently modulate diffusible and juxtacrine signaling between cells. Having cells in a grid also makes it much easier to track cells over several days. We believe that the ease of cell patterning with the BFC will encourage other researchers to use it in their studies.

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References

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