MICROPATTERNED COVERSLIPS

Materials included:

Micropatterned glass coverlips

Materials not included:

20-200 µL/100-1000 µL micropipette
Distilled water
PBS
Kimwipes
Parafilm
Plastic tweezers
Coverslips rack
6 well-plates or additional Petri dishes

Optional:

Coating solution (e.g. fibronectin)
Fluorescent-labelled protein (e.g. fibrinogen)

- → The micropatterned coverslips are sent inside standard 35 mm Petri dishes, patterns facing upwards.
- → To avoid contaminations, only manipulate the coverslips using aseptic techniques in a laminar flow hood.
- → The micropatterns consist of a thin layer of polymer bound to the surface of the glass. To avoid scratching this layer, pipette solutions onto the coverslips carefully and avoid touching the surface of the glass.
- → The timing of protein and/or cell incubation can vary depending on your needs. The protocol suggested by 4Dcell was optimized for HeLa cells. You may need to adapt this protocol for your cell type.
- → Incubation with an adhesion protein is optional. We suggest only using an adhesive protein if your cell type does not readily adhere to glass substrates. Several adhesive coating proteins can be employed if needed (fibronectin, laminin or collagen).

PROTOCOL

a. Open the individual plastic bag containing the Petri dish using aseptic technique in a laminar flow hood.

If coating the coverslips with an adhesion protein is necessary, proceed to step a.1. Otherwise, proceed directly to step b.

a.1. Optional: The coverslip is attached to the Petri dish, and the patterned side of the coverslip faces up. To remove it, fill the Petri dish with distilled water, and wait until the coverslip floats.

a.2. Optional: Remove the coverslip from the Petri dish and let it air dry. Remove the water from the Petri dish as well and let it air dry.

a.3. Optional: Pipette 100 µL of your adhesive protein on parafilm[1][2].

a.4. Optional: Carefully place the micropatterned side of the coverslip on the drop of fibronectin.

a.5. Optional: Incubate for 30 min to 1 hour at room temperature.

a.6. Optional: Rinse the coverslip with distilled water and allow it to air dry before proceeding to the cell seeding step. Once dried, place the coverslips back in the Petri dishes or in a 6 well-plate. The patterned side of the slide facing up.

b. Prepare a cell solution. For one coverslip we recommend 30 000 – 60 000 cells resuspended in up to 400 µL of cell culture medium[3].
c. Pipet 400 µL of cell suspension into each coverslip.
d. Carefully transfer the Petri dishes/6-well plates from the hood to the incubator[4].
e. Incubate the micropatterned coverslips containing the cells for 1 hour (or more, depending on cell type) at 37°C, 5% CO2.
f. Remove medium and unattached cells and replace with pre-warmed culture medium or sterile PBS[5].
g. Discard medium or PBS, add 2 mL of fresh pre-warmed culture medium per Petri dish or well and incubate cells overnight. Patterned cells can be observed under the microscope after several hours of incubation.

NOTES

[1] The fibronectin solution provided by 4Dcell has a concentration of 50 µg/mL. It can be adjusted according to the cell type. The incubation with fibronectin can be performed directly on the coverslips attached to the Petri dish (higher volume of protein solution is need to cover the whole glass coverslip).
[2] The patterns can be visualized using fluorescent fibrinogen or other fluorescent protein. The fibrinogen solution provided by 4Dcell has a concentration of 20 µg/mL.
[3] The seeding density is optimized for HeLa cells. The number of cells added and the adhesion or culture times could vary for other cell types. Make sure the cell suspension is thoroughly re-suspended into individual cells before pipetting into the wells. It is better to re-suspend cells that are at a confluency around 50% or less. High confluency of cells before re-suspension and seeding onto the plate will promote cell-cell adhesion and cell cluster formation, which is not suitable for single cell patterning.
[4] Avoid allowing the cell suspension to spread beyond the edge of the coverslip at this stage. This will ensure that the cell suspension remains on top of the coverslip and not in the surrounding area while the cells are attaching to the micropatterns.
[5] Removing the unattached cells will reduce cell clustering and bridging between micropatterns.