Micro-patterning on glass coverslip with deep UV light

(extended protocols can be obtained from the lab of Matthieu Piel at Institut Curie)

Material :

• UV lamp 185 nm (important : wavelength has to be less than 200 nm)
• Plasma cleaner (optional)
• Synthetic (!) Quartz mask with features (Delta Mask B.V.or Toppan Photomasks)
• Glass coverslips 22 mm (Marienfeld GmBH)
• PLL(20)-g[3.5]-PEG(2): poly-L-lysine-g-poly(ethyleneglycol), (standard PLL-g-PEG from SurfaceSolutions GmbH, Zurich), keep a stock solution at 1mg/mL in 10mM Hepes pH 7.4 at 4°C, use at 0.1mg/mL in 10 mM Hepes pH 7.4; The PLL-g-PEG is very stable and can be kept for a long time. It can even be reused, just sterile filter it after use.
• Fibronectin purchased from Sigma, reference F1141 or similar, aliquoted in 25-50 µL aliquots stored at - 20°C. Once diluted, it is stored at +4°C.
• Fibrinogen- A488 (green), - A594 (red), -647 (far-red) 1 mg/mL (optional)

Preparing the glass coverslips:

- sonicate coverslips in EtOH 70% for 5 minutes and air dry or plasma clean for 1 min (this step can be skipped);

- activate with the UV lamp for 5 minutes;

- incubate in 0.1 mg/ml PLL-g-PEG for 1 h. Put a drop of 50 to 200 µl PEG solution on a parafilm and put the activated face of the coverslip on top;

- wash 2x 5 min in PBS, then rinse 2x in H2O;

UV illumination:

- wear powder-free gloves;

- wash mask with 70% EtOH (use a soft tissue such as kimwipe for cleaning not to scratch the mask). Repeat washing steps if necessary;

- put the **quartz mask with its brown side facing the lamp** (use spacers to avoid contact with the bottom of the lamp box) and illuminate 5 min to activate the surface;

- put **4µl** of millipore H2O (for 22 mm coverslip) onto the brown side of the quartz mask (not on the silver one!) and put the coverslip with the pegylated side onto the water drop;

- use a plastic tip to press the coverslip against the mask and thereby spread the drop. **Attention: every resting air bubble will cause enlargment of underlying patterns!** This accounts also for air bubbles that arise due to evaporation during illumination. One solution to avoid evaporation can be to put a very large glass coverslip sealed with water onto the 22 mm coverslips;

- put the **quartz mask with its silver side facing the lamp** and illuminate 3-10 min. Minimal illumination time can vary for different cell types. In our hands, 3 min were sufficient for successful spreading of RPE1 cells, whereas HeLa cells needed at least 5 min;

- put 5 ml of water onto the coverslips and let it incubate for 5 minutes to detach the coverslip. Take off the coverslip with plastic forceps;

- incubate the illuminated surface with 25-50 µg/ml of fibronectin solution in 100 mM $NaHCO_3$ (pH 8.6) + 5 µg/ml of labelled fibrinogen for 1 h, RT. (use a drop of 100 µl solution on a parafilm) Using $NaHCO_3$ buffer is important. Covalent connection between activated PEG (carboxylic group) and deprotonated proteins aminogroup is favoured at basic pH. The use of labelled protein is useful to see the quality of the patterns made.

Plating of the cells :

- The cells can be detached with Trypsin or 0.02% EDTA. For some cells trypsin treatment will affect attachment and induce cell aggregation. You should test it with your cells. Centrifuge cells to get rid of the EDTA or trypsin. Resuspend cells using a 1 mL pipette to separate individual cells. Add 100.000 to 200.000 cells for a 25 mm coverslip.

- Wash unattached cells 20-30 min (depending on the cell type) after plating with equilibrated hot medium (to equilibrate a medium, leave it in a flask allowing gas exchanges in the incubator). To wash add medium from one side and aspirate from the other side in the same time.

- HeLa cells should be spread on the patterns 1 hour after plating.

Additional reading:

Théry and Piel. “Adhesive micropatterns for cells: a microcontact printing protocol”.

Cold Spring Harb Protoc. 2009 Jul;2009(7):pdb.prot5255. doi: 10.1101/pdb.prot5255.

Ammar Azioune, Nicolas Carpi, Qingzong Tseng, Manuel Théry, Matthieu Piel. “Protein micropatterns: a direct printing protocol using deep UVs”. Methods Cell Biol. 2010; 97:133-46. doi: 10.1016/S0091-679X(10)97008-8.