Materials needed

UV lamp (λ = 254 nm, P = 7 mW/m²) under a fume hood or linked with an ozone filter. (UVO Cleaner, Jelight).

Synthetic Quartz mask with features (Toppan / 4DCells / JD Photodata).

Glass coverslips 25 mm (or any size you wish to use). [My reference: Marienfeld 0111650]

PLL(20)-g[3.5]-PEG(2): poly-L-lysine-g-poly(ethyleneglycol). Keep a stock solution at 0.5 mg/mL in 10 mM Hepes pH 7.4 at +4°C. Use at 0.1 mg/mL in10 mM Hepes pH 7.4. The PLL-g-PEG is very stable and can be kept for months (Surface Solutions / Jemken). Note: when diluting the stock solution from 0.5 mg/mL to 0.1 mg/mL:

Prepare a small volume that can be used in the coming weeks.

Filter it with a 0.2 µm sterile filter (to remove potentially big impurities from the solution, which would affect the passivation homogeneity). [Filter spec. : 0.2 µm, sterile, non-pyrogenic, hydrophilic (Clearline 146560)].

Fibronectin from bovine plasma. Aliquoted in 25 µL at 1 mg/mL aliquots stored at-20°C. Once diluted, it is stored at +4°C. Reference F1141 (Sigma-Aldrich).

Fibrinogen-{Alexa488 (green), Alexa594 (red), Alexa647 (far-red)} (Invitrogen) at 1.5mg/mL in NaHCO3 buffer (100mM, pH 8.3, powder from Sigma Aldricht ref. S-6297).

For chamber manufacturing (optional) :

Aquarium glue (Zolux Silicone SA 500).

35mm petri dishes.

Epilog engraver laser cutter (accessible in the PMMH lab by Joseph).

Step 1: Coverslip passivation with PLL-Peg

Clean N round glass coverslips 25mm with isopropanol and dry them with a Kimtech or compressed air. At least one of the two surfaces of each coverslip has to be perfectly clean.

Put these coverslip in the plasma oven, 2' to make the vacuum, 3' exposition to plasma. To support the coverslip in the oven, I like to use a porcelain slide-holder.

On a parafilm sheet, put N 50µL drops of filtered PLL-Peg solution [0.1 mg/mL in 10 mM Hepes pH 7.4, filtered with a 0.2µm sterile filter]. Place each of the plasma-cleaned coverslip over one of these droplets, so that the whole bottom-surface of each coverslip rests on an homogeneous layer of PLL-Peg solution.

Let incubate for 40 minutes.

Remove each coverslip with a tweezer and gently rinse them in a milliQ water bath. Then dry them by absorbing the water with a Kimtech (no direct contact with the coated surface). At this point they can be stored in a dish, with the PLL-Peg coated face up. The coating should be stable over a long time.

Step 2: Micropattern burning with a mask and deep-UV

Clean the metallic surface of the mask with isopropanol + Kimtech / compressed air, and then by exposing it to deep-UV for 10', metallic face toward the UV source. (This can be done during the 30' PLL-Peg incubation of step 1).

Put a small droplet of milliQ water on each pattern location of the mask (on the metallic face). The volume of water depends of the coverslip size; for 25mm round coverslips, 8~12µL of water is a good volume. Take your PLL-Peg coated coverslips, and place each of them on the water droplets so that a uniform water layer is formed. The less air bubble the better. At this point the water layer should be too thick: the coverslips are floating on it. With a compressed air gun, blow air on the coverslips from the top, to push them against the mask and remove the excessive water simultaneously. When the layer is thin enough, iridescence will appear and cover the whole interface.

Put the mask under UV-light for 10', with off course the non-metallic face toward the UV source, so that the light properly go through the mask before reaching the coverslips.

To remove the coverslips, pour a puddle of milliQ water on the mask's face with the coverslip, so that they slowly detach from it. It might be a bit long, and require some help with a plastic tweezer / pipet tips. Note that sliding the coverslip to the edge of the mask will work but there is a risk of scratching the PLL-Peg, and thus damaging the pattern.

Dry the coverslips with a Kimtech. Again, at this point they can be stored in a dish, with the PLL-Peg coated face up. The patterning should be stable over a long time.

Step 3: Optional - Experimental chamber manufacturing

To obtain chambers similar to fluorodishes with the patterning on the glass surface.

Take 35 mm petri dishes, and cut holes of 20 mm in their base. Some do it with a dremmel drill, I think the best solution is a laser cutter (you can do batch processing and achieve better precision).

Clean thoroughly the covers and bases of the cut petri dishes with ethanol and dry.

Put some aquarium glue in a syringe (~1,5 mL of glue for 4 dishes). Take each dish base and apply a thin line of glue around the 20 mm hole (on the bottom of the base), then put a PLL-Peg patterned coverslip on top and very gently press with a plastic tweezer / pipet tips to spread the glue. You should observe a continuous layer bonding the coverslip to the dish, effectively sealing the hole.

Let the glue dry for 12h.

Step 4: Micropatterning fibronectin

Prepare a solution of fibronectin (10~20 µg/mL) + fibrinogen-fluo (2~5 µg/mL) in NaHCO3. Filter with 0.2 µm filter.

Depending of the choice between bare coverslip and fluorodish-like chambers:

For bare coverslip, put droplets of 100 µL of protein solution on a clean parafilm sheet, and put each coverslip on a droplet, patterned face down off course. Let incubate 30' and pour a puddle of PBS to detach the coverslip.

For fluorodish-like chambers, cut discs of parafilm slightly smaller in diameter than the holes in the bottom of the chambers. Put 125 µL of protein solution in each chamber and spread the drop by covering it with a parafilm disc. Let incubate for 30' and pour 2 mL of PBS in each chamber to remove the parafilm disc by making it float. Note: Do not attempt to remove the parafilm disc with a tweezer before filling the chamber with PBS, it might make the pattern very dirty.

Bonus: Cell adhesion

As a reference for future experiments, here is my protocol to make 3T3 fibroblasts adhere on a pattern of 20 µm fibronectin discs.

\*\*TBC\*\*

Leads for improvements

In step 1, using ethanol instead of isopropanol might be enough.

In step 1, the 5' exposition to plasma might be excessive.

If this protocol is performed in a non-sterile environment, between step 3 and step 4, exposing the coverslip / chambers to UV for a small time might help to sterilize the potentially contaminated objects.