Micropatterning on silicon elastomer (PDMS) with deep UVs

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Method Article

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Abstract

This protocol describes a technique to imprint adhesive micropatterns on silicon elastomers like PDMS \(poly-dimethyl siloxane). The micropatterns are stable for days \(depending on the cell type) and regions outside the patterns prevent cell attachment. This technique is fast and easy and can be useful to combine cell stretching and micro-patterning.

Introduction

This protocol explains how to make high resolution adhesive micropattens of proteins on silicone elastomer like PDMS. The first step is to passivate the surface with polylysine grafted polyethyleneglycol \(PLL-g-PEG). This coating is then irradiated with deep UV through a photomask. Deep UVs will destroy PEG chains and allow proteins to bind. Cells will then adhere on the patterned proteins. This protocole is similar to patterning on glass, except it brings a tip to solve the main problem arising with PDMS substrates, which is binding of the PEG to the surface. 

Reagents

• Silicone membranes: Gelpak \(exists in various thickness) • PLL\(20)-g\[3.5]-PEG\(2): poly-L-lysine-g-poly\(ethyleneglycol), \(PLL-g-PEG from SurfaceSolutions GmbH, Zurich). Stock solution at 1mg/mL in 10mM Hepes pH 8.6 at 4°C, use at 0.5 mg/mL in 10 mM Hepes pH 8.6; The PLL-g-PEG is stable and can be kept for months. • N-\(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride \(EDC) from Sigma \(ref : 03450). Unusable after 6 months. Keep at -20°C. • N-Hydroxysulfosuccinimide sodium salt \(Sulfo-NHS) from Sigma \(ref : 56485), kept at -20°C. Let it get back to room temperature before opening the vial as it is very hygroscopic and must be protected from water. • 2-\(N-Morpholino)ethanesulfonic acid, 4-Morpholineethanesulfonic acid \(MES) from Sigma \(ref : M3671) • Sodium Chloride \(NaCl) from Sigma \(ref : S7653) • Fibronectin from bovine plasma from Sigma \(ref F1141), aliquoted in 25 µL aliquots stored at -20°C. Once diluted, it is stored at +4°C. • Vacuum silicon grease \(GE Bayer Silicones, ROTH ref 0856.1) • Ethanol 70% • Optional : Fibrinogen- A488 \(green), - A594 \(red), -647 \(far-red) 1 mg/mL \(Invitrogen)

Equipment

• UV ozone oven 185 nm \(important : wavelength has to be less than 200 nm) under a hood or equiped with ozone catalyser \(UVO cleaner, model 342-220, Jelight). • Synthetic Quartz mask with features \(Delta Mask, Toppan, Selba Tech)

Procedure

PDMS passivation : 1. Wash the PDMS in Ethanol 70% for 15 minutes and dry it under air flow. 2. Illuminate the PDMS with the deep UVs lamp for 5 minutes.The distance with the lamp should be about 5

cm for the Jetlight UVO cleaner. 3. During the illumination, weight the EDC/NHS. For 1 mL solution use 11.5 mg NHS and 19.2 mg EDC. 4. Dissolve the powder in a 0.05 M MES + 0.5 M NaCl buffer at pH 6.0. 5. Cover the PDMS substrate with the EDC/NHS solution. 6. Incubate 15 minutes at room temperature in the dark. 7. Rince the surface with PBS and then H~2O. 8. Cover the surface with 0.5 mg/mL PLL-g-PEG solution in Hepes buffer 10 mM ph 8.5. 9. Incubate 3 hours or overnight at room temperature, in humid chamber to avoid evaporation. 10. Wash with H~2O. The passivated surfaces can be kept at +4°C several days in water. **UV illumination :** \(wear nitrile gloves) 1. Wash mask with 70% Ethanol \(use a soft tissue for cleaning not to scratch the mask). Repeat washing steps if necessary. 2. Put the quartz mask with its metal side \(bearing the features) facing the UV lamp 3. Illuminate 5 min to clean the surface and make it hydrophilic. 4. Add the passivated PDMS substrate on the mask \(on the metal side) avoiding bubbles. 5. Put the quartz mask with its quartz side facing the lamp \(PDMS on bottom). 6. Illuminate 5-10 min Minimal illumination time can vary for of different cell types. In our hands, 5 min were sufficient for successful spreading of RPE-1 cells. 7. Carefully and very slowly peel off the PDMS from the mask. Add water to facilitate this crucial step \(PLL-g-PEG can be left on the mask and therefore diminish the quality of the patterns.) 8. Incubate the illuminated surface1 h at room temperature with 25 µg/mL of fibronectin solution in 100 mM $NaHCO_3$ \(pH 8.5) . To visualize the patterns, you can add 5 µg/mL of labelled fibrinogen or fibronectin. Use a drop of 100 µl solution on a parafilm. $NaHCO_3$ basic buffer favors protein binding on the illuminated regions. **Plating of the cells :** 1. Collect cells preferably using EDTA 0,02% in PBS and NOT trypsin. This is particularly important for HeLa cells. Using EDTA allows faster rebinding of the cells in the patterns, which is important to avoid cells clustering before adhering on patterns. 2. Rince EDTA solution with pre-warmed and pH equilibrated culture medium \(important for cells to rebind fast). 3. Resuspend cells using a 1 mL micropipette to seperate them well. 4. Add 100.000 to 200.000 cells for a 25 mm coverslip or 6 well plate well. Use a small volume \(for exemple 500 µL for a 35 mm well or dish). This will allow cells to fall fast on the surface and to remain homogeneously spread. Leave the dish in the incubator without moving it. 5. Wash unattached cells 20-30 min \(depending on the cell type) after plating with equilibrated hot medium. To wash, add medium from one side and aspirate from the other side at the same time to create a gentle flow over cells. Cells should be spread on the patterns 1 hour after plating. **Assembly on a stretching device** 1. Attach the patterned silicone membrane to the device 2. Cut a square piece of PDMS. Empty the center to obtain a four walled chamber. 3. Use silicone grease to bind the chamber on top of the patterned silicone membrane. 4. Plate the cells as described above 5. The chamber can be closed with a coverslip to avoid evaporation. 

Timing

Initial coating : 3 hours Illumination : 10 mins Incubation of protein and washing : 1h30 Plating the cells : 30 min **Total time : 5 hours**

Troubleshooting

The cells are spreading everywhere : This is the most common problem with this protocole. The two main reasons for this problem are: - the PEG is removed from the silicone membrane when peeling it off from the mask \(check your peeling technique by comparing a passivated membrane you have not placed on the mask and one you have placed on the mask, both should repell cells). - the crosslinking of PEG is bad, due to poor reagents. Sulfo-NHS and EDC are not very stable products and should be changed if such problem occurs. **No cells are attaching the surface :** The fibronectin might not be attached to the surface. This can be due to insufficiant illumination of the PEG with deep UVs. Increase illumination time and/or fibronectin incubation time \(or concentration). Also check your protein solution and buffer \(the protein buffer is important and should be at the right pH). **Cells do not spread fully on the patterns :** Same as above, increase fibronectin concentration / incubation time / illumination time. Your cells might also prefer another adhesion protein. It is also important, if you want single cells to spead over the entire pattern, to match the pattern size with the size of the cells you use, this can vary a lot. For exemple HeLa cells like surfaces around 700 μm^2 while RPE1 prefer around 900 μm^2. Check Fink et al., Lab On Chip, 2007 for more detailed troubleshooting, also for other patterning techniques. Note that there are many protein patterning techniques, and that this one might not be optimal for your cells or application. We have been writing several technical papers on micropatterning, with different approaches. They are listed in the references \(directly related to this technique refs 5 and other techniques 1-4,6). We do not wish to review all the potential techniques so we do not refer to papers from other labs \(but many are cited in our papers). It is worth checking in particular articles from the lab of Christopher Chen who contributed many techniques for micropatterning with alternative approaches.

Anticipated Results

A good result is about 30% of patterns with a single cell. Cells should take the shape of the micropattern. No cell should spread outside the micropatterns. Cells should stay attached and confined at least 24 hours but can be kept on patterns up to several days depending on the cell type. 

References

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Figures

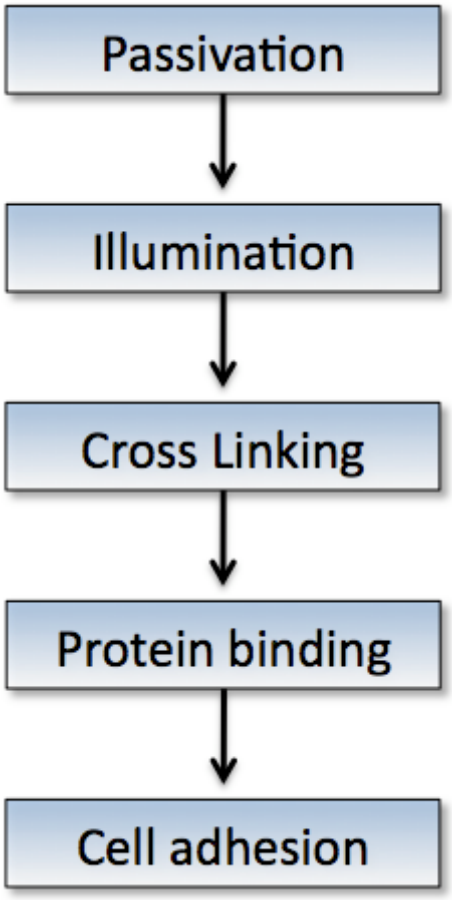

Figure 1

The steps of the protocol This figure is the flowchart of the protocol.

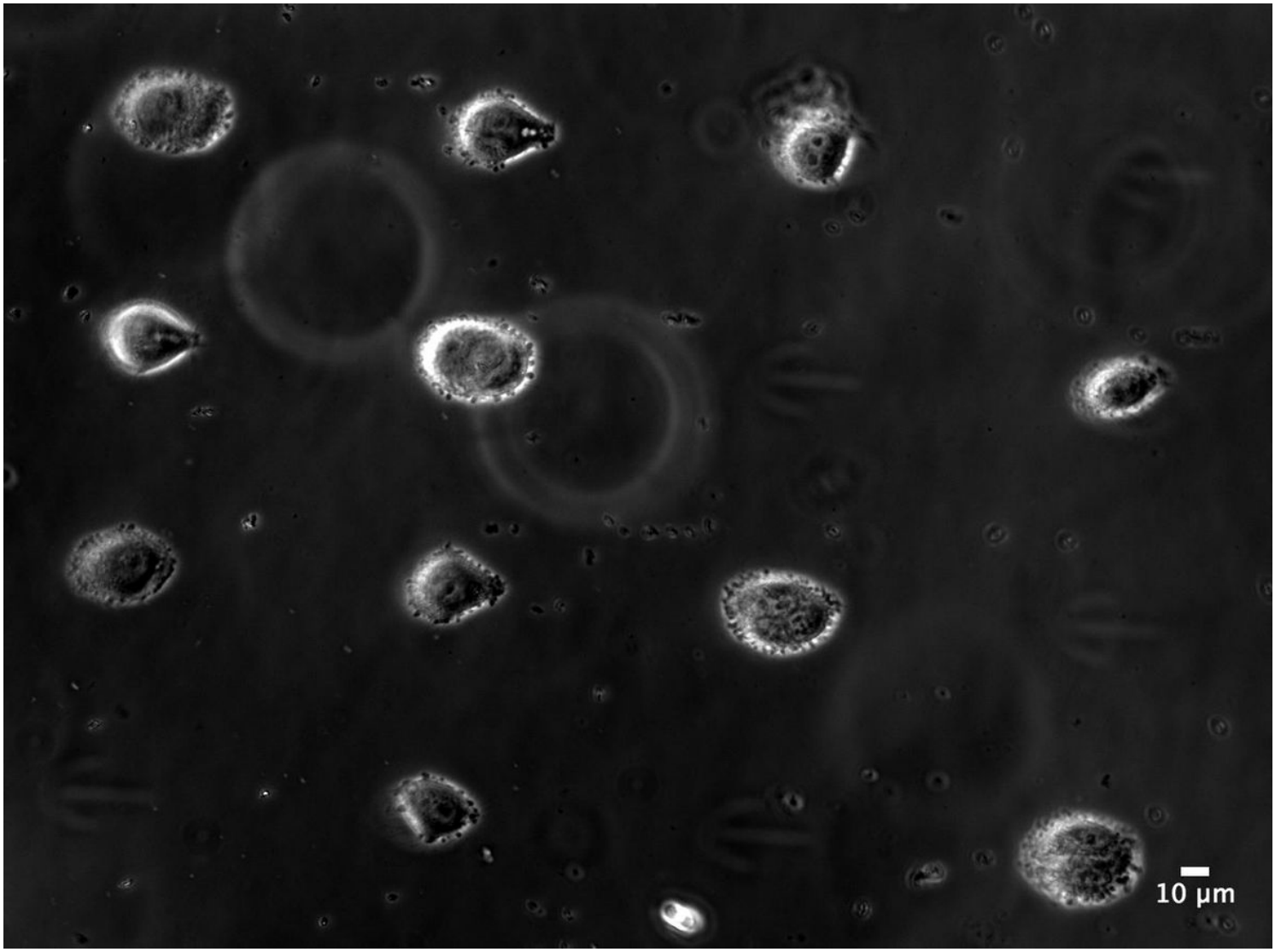

Figure 2

Patterned HeLa cells HeLa cells patterned on PDMS with fibronectin.

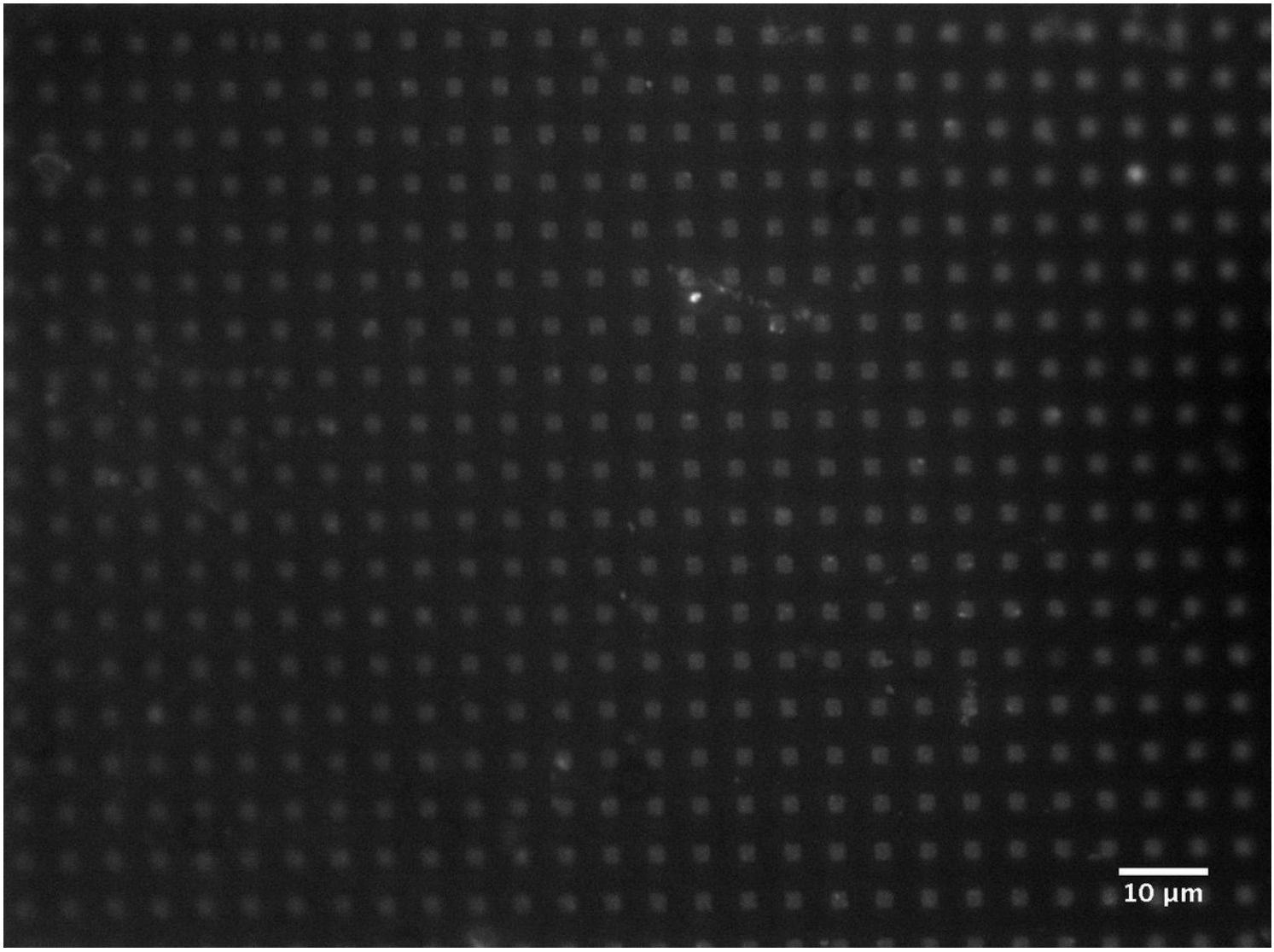

Figure 3

Fluorescent micropatterns Micropatterns showing the high resolution of the technique. Patterns appear fuzzy because of the focus, not the technique.

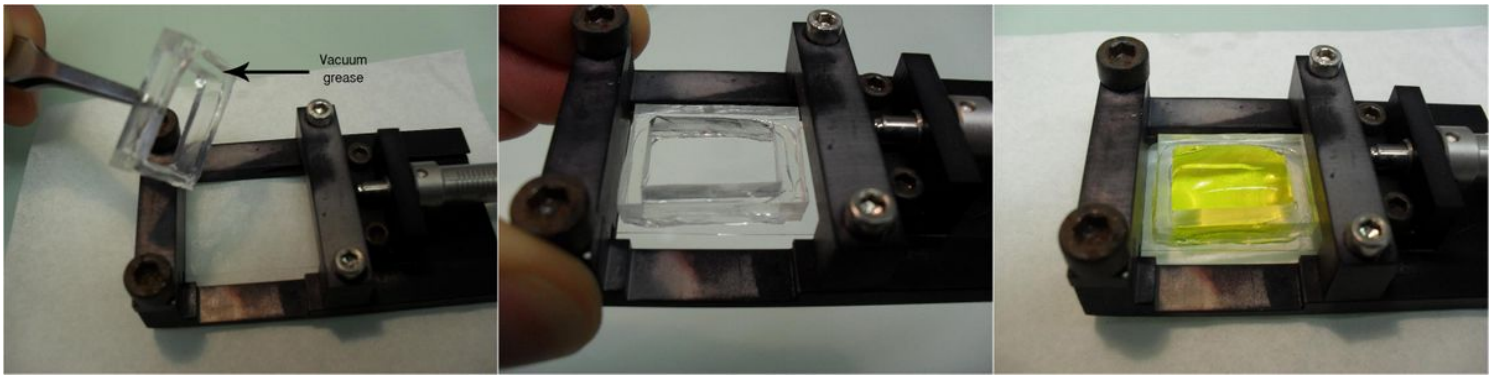

Figure 4

PDMS pool The PDMS pool has vacuum grease under, and is placed on the PDMS substrate in order to be able to put medium inside. Cover with a coverslip to avoid evaporation.