Micropatterning on glass with deep UV

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Method Article

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Abstract

This protocol describes a method to print micropatterns on glass with extra-cellular matrix proteins to promote cell adhesion. The non-adhesive part is made with polylysine grafted polyethyleneglycol \(PLL-g-PEG). This technique is reproducible, cheap, fast and can achieve high resolution \(~1 µm).

Introduction

This protocol explains how to make high resolution adhesive micropattens of proteins on glass, the primary application being to pattern adherent cultured cells. It has three simple steps: 1) incubate the glass substrate with polylysine grafted polyethyleneglycol \(PLL-g-PEG). 2) irradiate the substrate with deep UV through a photomask. deep UV will destroy PEG chains to allow proteins to bind. 3) incubate the patterned substrate with proteins. Cells will then adhere only to the part wich have been illuminated and where adhesion proteins adsorbed, while the rest of the surface prevents protein adsorption and cell adhesion. Note that there are many micropattering techniques available and that none provides a universal solution. The technique presented here is fast and easy but might have a lower robustness than more complicated techniques. 

Reagents

• Glass coverslips \(Marienfeld GmBH) • PLL\(20)-g\[3.5]-PEG\(2): poly-L-lysine-g-poly\(ethyleneglycol), \(SurfaceSolutions GmbH, Zurich), 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; PLL-g-PEG is stable and can be kept for months, it can even be reused if you need to use a large volume, just sterile filter it before and after use. • Fibronectin \(Sigma, reference F1141), aliquoted in 25 µL aliquots stored at -20°C. Diluted in NaHCO~3 10 mM pH 8.5, stored at +4°C. • 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

Glass passivation : 1. Wash glass coverslips in Ethanol 70% for 1 minute and dry them with absorbant paper. 2. Illuminate the coverslips with the deep UVs lamp for 5 minutes.The distance with the lamp should be 5 cm for the Jelight UVO cleaner. 3. Put a drop of 50 to 200 µl \(depending on the coverslip size) of a 0.1 mg/mL PLL-g-PEG solution on a parafilm and put the coverslip on top, activated face in contact with the PEG. Incubate 1 hour at room temperature, in humid chamber to avoid evaporation. 4. Wash 2 min in PBS, then rinse 2 times 2 min in H2O **UV illumination :** \(wear 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. Put 3µl of millipore H2O \(for 25 mm coverslip) on the metal side of the mask 5. Place the coverslip with the pegylated side on the water drop. 6. Use a plastic tip to press the coverslip against the mask and remove the excess water Caution: any air bubble will cause enlargment of underlying patterns \! This is also true for airbubbles that arise due to evaporation during illumination. 7. Put the quartz mask with its quartz side facing the lamp \(coverslips on the opposite side). 8. Illuminate 3 to 10 min Minimal illumination time can vary for different cell types. In our hands, 3 min were sufficient for successful spreading of RPE-1 cells, whereas HeLa cells needed at least 5 min, as they are more sensitive to remaining PEG molecules. 9. After illumination, put 5 mL of water on the coverlip and leave until the coverslip spontaneously floats. 10. Take the coverlip carefully to avoid scratching the mask with the tweezer or use plastic tweezers. 11. 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.

Timing

PEG coating : 1 hour \(coverslips can be stored for a week after that stage) Illumination : 10 mins \(coverslips can also be stored for a week after that stage) Incubation of protein and washing : 1h20 \(coverslips should be used in the following 2 or 3 days after that step and kept in PBS at +4°C) Plating the cells : 30 min **Total time : 3 hours**

Troubleshooting

The cells are spreading everywhere : This is probably due to a bad passivation, the PLL-g-PEG step hasn't been done right or your PEG solution is bad \(it could be too old, or the pH might be wrong). Check if the cells don't attach on a coverslip with just PLL-g-PEG coating. **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 1,2 and other techniques 3-6). 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 \(see Figure 2). 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

1. Azioune, A. et al. Protein micropatterns: A direct printing protocol using deep UVs. Methods Cell Biol. 97:133-46 \(2010). 2. Azioune, A. et al. Simple and rapid process for single cell micro-patterning. Lab Chip. 2009 Jun 7;9:1640-2 \(2009). 3. Fink, J. et al. Comparative study and improvement of current cell micro-patterning techniques. Lab Chip. 7:672-80 \(2007). 4. Théry, M. and Piel, M. Adhesive micropatterns for cells: a microcontact printing protocol. Cold Spring Harb Protoc. 2009:pdb.prot5255 \(2009). 5. Azioune, A. et al. Robust method for high-throughput surface patterning of deformable substrates. Langmuir. 27:7349-52 \(2011). 6. Tseng, Q. et al. A new micropatterning method of soft substrates reveals that different tumorigenic signals can promote or reduce cell contraction levels. Lab Chip. 11:2231-40 \(2011).

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Figures

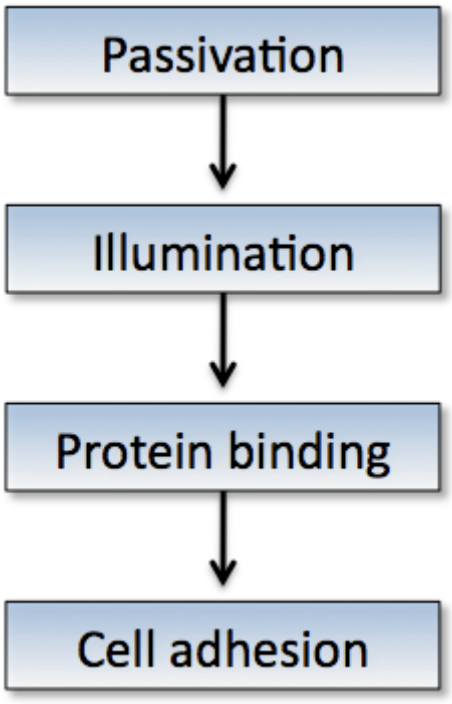

Figure 1

Flow diagram This figure presents an overview of the steps involved in the protocol.

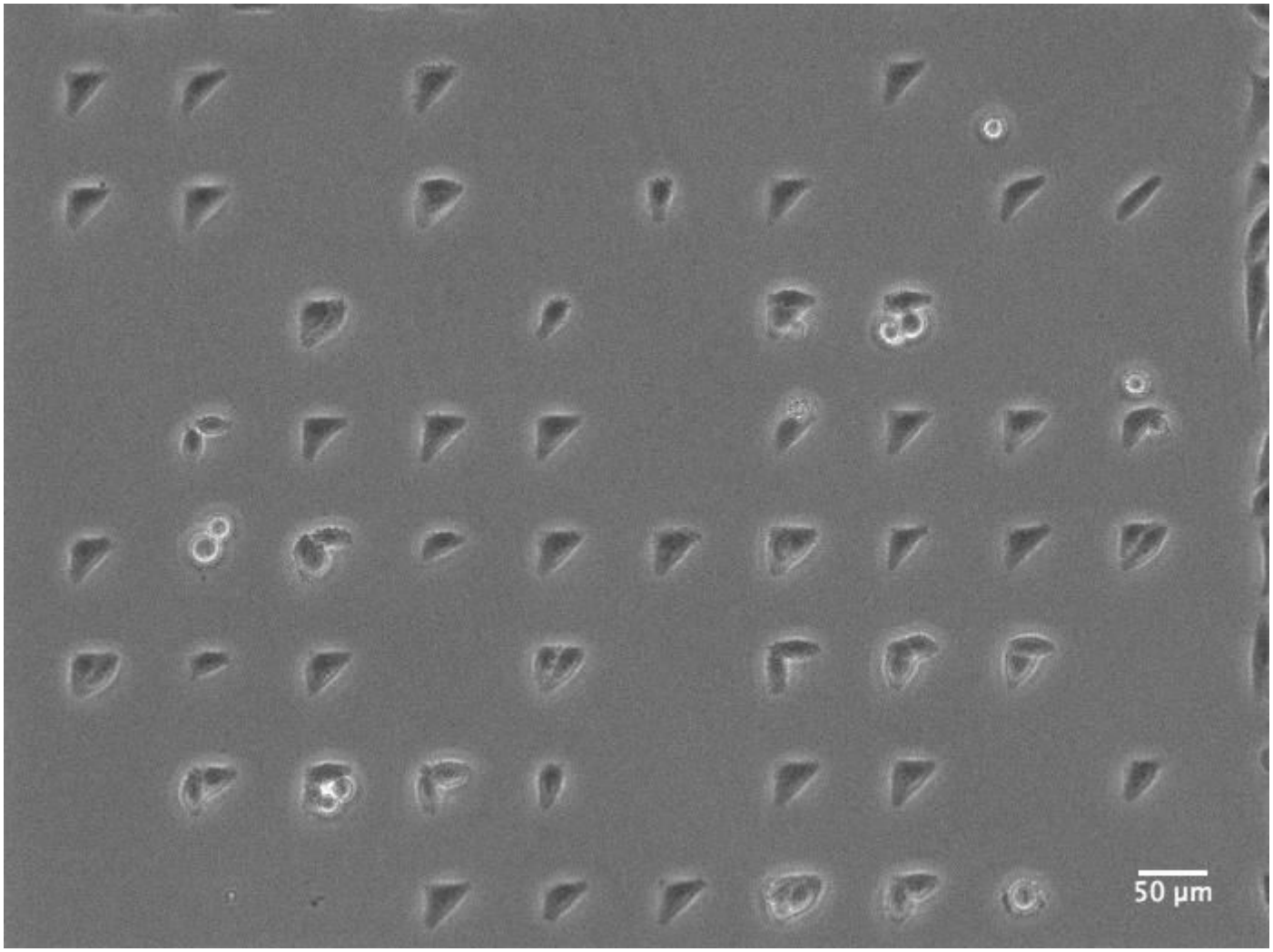

Figure 2

Patterned HeLa cells HeLa cells patterned on glass with fibronectin.

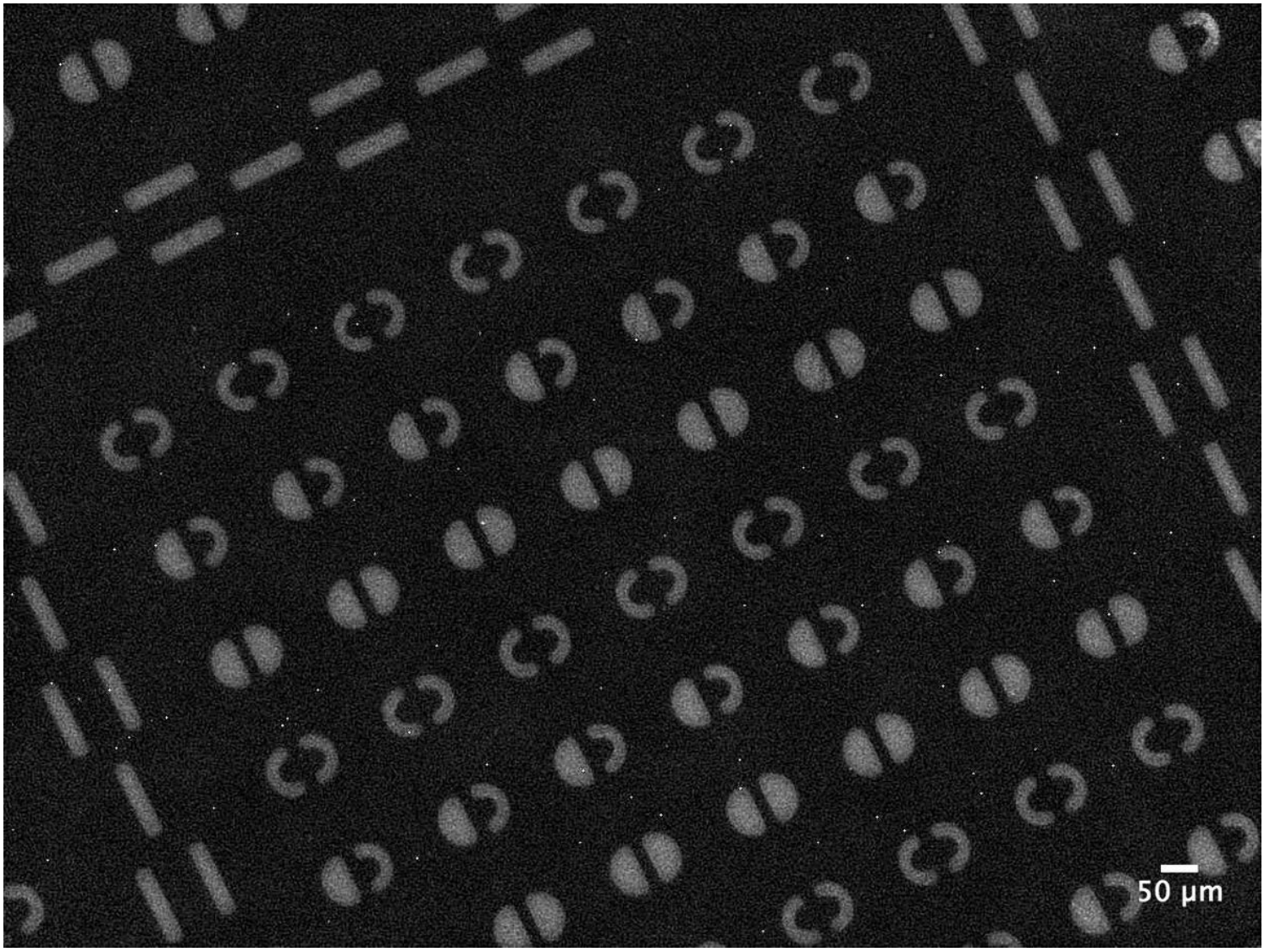

Figure 3

Fluorescent micropatterns Micropatterns visualized with fluorescent fibronectin.