MICROPATTERNING CELL ADHESION ON POLYACRYLAMIDE HYDROGELS

Materials

1. Freshly prepared Bind-silane working solution. Mix 950 μl of ethanol (95%; ACS/USP grade) and 50 μl of acetic acid with 3 μl of Bind-silane (Sigma-Aldrich, St. Louis, MO).

2. Coverslips (45 mm x 50 mm, No. 1)

3. Diamond-tip pen

4. Bunsen burner

5. SPR-220.3 positive photoresist (MicroChem, Newton, MA).

6. Coverslips (45 mm x 50 mm, No. 2)

7. Spin coater (e.g. Spin Processor from Laurell, North Wales, PA). An inexpensive low-speed tabletop centrifuge may be modified to hold coverslips for spin coating (Guo & Wang, Cold Spring Harb Protoc 2011(3):prot5582).

8. Heating block or plate with precise temperature control for 115 °C.

9. UV source for i-line (365 nm). An inexpensive UV station with relatively uniform exposure may be constructed by mounting a high flux UV LED (Opto Technology, Inc., Wheeling, IL) over an orbit shaker where photoresist-coated coverslip is placed. The setup is shown in (Guo & Wang, Cold Spring Harb Protoc 2011(3):prot5582).

10. Photomask with the desired pattern. Glass photomasks (e.g., from Advance Reproductions Corp, North Andover, MA) are required for a resolution of pattern better than 10 μm. Otherwise, the mask may be ordered as an inexpensive transparency film (e.g. from CAD/Art Services, Bandon, OR).

11. Microposit Developer MF-319 (MicroChem).

12. Glass Petri dishes.

13. Orbit shaker in a chemical fume hood.

14. Sylgard® 184 Silicone Elastomer Kit, including Base and Curing Agent (Dow Corning Corporation, Midland, MI).

15. Heating block or incubator with precise temperature control for 70°C.

16. Disposable beakers.

17. Gelatin or ECM protein such as collagen or fibronectin.

18. Sodium m-periodate (Sigma-Aldrich).

19. Glass coverslips (25 mm × 25 mm, No. 1).

20. Acrylamide.

21. Bisacrylamide.

22. HEPES: 200 mM, pH 8.5.

23. Ammonium persulfate (APS), freshly prepared 10% (W/V) solution.

24. N, N, N', N'-Tetramethylethylenediamine (TEMED).

Procedure

1. Mark one side of 45 mm x 50 mm, No.1 coverslips with a diamond-tip pen. Pass the coverslip over the inner flame of a Bunsen burner with the marked side facing the flame. The plasma in the flame increases the hydrophilicity of the glass surface. Allow the coverslips to cool to room temperature.

2. In a fume hood, apply ~30 µl of Bind-silane working solution onto the flamed side of coverslips and smear it evenly with the pipet tip. Remove excess Bind-silane with Kimwipes®. Allow the Bind-silane to react for 3 min.

3. Rinse treated coverslip surfaces with ethanol and wipe with Kimwipes® to remove any residual Bind-silane solution. Allow to air-dry. (*see* Note 1).

4. Pass coverslips (45 mm x 50 mm, No.2) over the inner flame of a Bunsen burner with the marked side facing the flame. Allow the coverslip to cool to room temperature.

5. In a fume hood with a spin coater set at 5,000 rpm for 30 s, spread 180 µl positive photoresist SPR-220 uniformly across the flamed side of the coverslip.

6. Bake the coverslips at 115^{o}C for 90 s on a heating block. Allow the coverslips to cool to room temperature.

7. Place the photomask over the coverslip and expose the assembly to 365 nm UV light. The exposure depends on the intensity of the light source. Using a high flux UV LED at a distance of 3 cm from the coverslip on an orbit shaker, the optimal exposure is around 45 s (*see* Note 2).

8. Bake the coverslip at 115^{o}C for 90 s on a heating block. Allow the coverslip to cool to room temperature.

9. Immerse the coverslip in Microposit Developer MF-319 in a glass Petri dish, placed on an orbit shaker inside a chemical fume hood, for approximately 45 s. Optimal timing and mixing conditions are affected by the exposure condition and should be controlled carefully (*see* Note 3).

10. Rinse the coverslip extensively in deionized water and allow to air-dry. The pattern on SPR-220, which serves as the molding for PDMS stamps, should be visible (*see* Note 4).

11. Weight out approximately 5 g of Sylgard® 184 Silicone Elastomer Base, add 1/10 volume (V/W) of the curing agent, and mix thoroughly. Degas for 30 min using house vacuum to remove air bubbles.

12. Incubate the coverslip covered with Sylgard at 70°C on a heating block or in an incubator for at least 1 h.

13. Dilute gelatin to a final concentration of 0.1% (W/V) in PBS, or Type I collagen to a final concentration of 0.01% (W/V) in 50 mM sodium acetate (pH 4.5), or fibronectin to a final concentration of 0.001% (W/V) in PBS.

14. While mixing, slowly add solid sodium m-periodate to the protein solution to reach a final concentration of 3.6 mg/ml. Allow the reaction to proceed for 30 min at room temperature (*see* Note 5).

15. Pass a 25 mm × 25 mm coverslip over the inner flame of a Bunsen burner. Allow the coverslip to cool to room temperature.

16. Pipet approximately 200 μl of the activated protein solution onto the surface of the stamp, incubate at room temperature for 30 min, then remove excess solution by blowing with a stream of nitrogen gas.

17. Prepare the acrylamide solution with desired concentrations of acrylamide and bisacrylamide in 10 mM HEPES. Typically 5% (W/V) acrylamide and 0.1% (W/V) bisacrylamide are used for the measurement of traction stress of fibroblasts. Degas with house vacuum for 20 min.

18. Press the stamp against the 25 mm × 25 mm coverslip for 5 min (*see* Note 6).

19. Add 0.006 volume of 10% APS and 0.004 volume of TEMED to the acrylamide solution and mix quickly and briefly to initiate the polymerization reaction.

20. Pipet 30 μl of the polymerizing acrylamide solution onto a Bind-silane-activated coverslip.

21. Without any delay, remove the stamp from the 25 mm × 25 mm coverslip and place the coverslip, stamped side facing down, on the polymerizing acrylamide solution.

22. Let acrylamide polymerize to completion for 15-20 min at room temperature.

23. Peel off the top coverslip carefully with a razor blade. Cover the hydrogel surface immediately with PBS to prevent drying (*see* Notes 7 and 8).

Notes

1. Activated coverslips may be stored in a desiccator at room temperature for at least 3 months.

2. Tight contact between the photomask and photoresist is critical if the light source is not well collimated. A simple method involves placing the photomask over the coverslip and

sandwiching them between two pieces of glass plates with paper clamps to ensure tight contact. In addition, it is critical to make sure that the patterned side on the photomask is facing the coverslip. For illumination with a less-than-uniform light source, the assembly of photomask and coverslip may be placed on an orbit shaker (Guo & Wang, Cold Spring Harb Protoc 2011(3):prot5582). Rotation at about 70 rpm during exposure creates a uniform average illumination across the surface. The exact exposure time depends on the optical condition and must be calibrated for each setup.

3. Developing in tetramethylammonium hydroxide (TMAH; 2.45% in 0.1% Triton X-100) generates results comparable to those using Microposit Developer MF-319.

4. To generate polyacrylamide substrate uniformly conjugated with ECM, simply use a clean coverslip to make a flat, pattern-less PDMS stamp.

5. Treatment with periodate causes vicinal diols in the sugar moieties of ECM proteins to undergo a ring-opening reaction, forming two aldehyde groups, which are capable of copolymerizing with acrylamide thus directly incorporating the ECM protein into the hydrogel. After the reaction, periodate can be removed with dialysis or a spin column. Activated proteins may be stored at -20 or -80 ºC for months.

6. The contact between the stamp and the coverslip should be even to ensure efficient transfer of the patterned protein. Even contact may be achieved by gently rolling a pencil back and forth over the PDMS stamp in two criss-cross directions. The quality of contact may be checked by looking at the glass-PDMS interface from different angles. Colorful interference fringes appear at the interface if the contact is good.

7. ECM-micropatterned hydrogel substrate should be sterilized using UV irradiation before inoculation with cells.

8. Non-glycosylated proteins may be patterned using a similar procedure. The protein is stamped onto the coverslip as described above without activation. Acrylamide solution is prepared with the incorporation of acrylic acid-N-hydroxysuccinimide, which reacts with protein lysine residues, at a final concentration of 0.05 mg/mL

Reference

Rape, A.D., Guo, W.-H., and Wang, Y.-L. (2011) The regulation of traction force in relation to cell shape and focal adhesions. *Biomaterials* 32:2043-2051.