PREPARATION OF POLYACRYLAMIDE SUBSTRATES

Materials

1. No.1 coverslip, 45x50 mm rectangular and 22 mm circular.

2. NaOH, 0.1 N, 100 ml.

3. 3-aminopropyltrimethoxy silane.

4. PBS, 500 ml.

5.glutaraldehyde, 0.5%. Mix 357 ul of 70% glutaraldehyde with 50 ml of PBS. Keep the 70 % stock tightly sealed in zip bags in a closed container at 4°C.

6. HEPES, 1 M, pH 8.5, 1 ml and 50 mM, pH 8.5, 500 ml. Use at room temperature.

7. Fluorescent latex beads, 0.2 um diameter.

8. Acrylamide (40%, Bio-Rad) and Bis (2%, Bio-Rad).

9. Ammonium persulfate (Bio-Rad) solution, 10 mg in 100ul distilled water. Prepare immediately before use in step 10.

10. TEMED (Bio-Rad).

11. sulfo-SANPAH (Pierce), 0.5 mg/ml in 50 mM HEPES pH 8.5, need 400 ul per dish. PREPARE IMMEDIATELY BEFORE USE IN STEP 15. Handle sulfo-SANPAH in the dark. Weigh the appropriate amount, add 1 ul DMSO per mg of sulfo-SANPAH. While vortexing, add 50 mM HEPES at room temperature to obtain the final concentration.

12. Protein solution for coating the substrate. Use type I collagen (10 mg/ml stock), at 0.2 mg/ml (40 ul + 2 ml PBS), or fibronectin at 10 ug/ml in a volume of 2 ml.

Procedure

1. Mark one side of a #1 cover slip with a diamond tip pen. Pass the marked side over the inner flame of a Bunsen burner.

2. Place the cover slip, flamed side up, on a test tube rack. Smear the surface with 0.1 N NaOH in the hood and allow the surface to air dry.

3. Smear the dried surface with 3-aminopropyltrimethoxy silane, wear gloves and do this in the hood. Incubate at room temperature for 5 minutes.

4. Collect the cover slips in a pan. Wash with distilled water on a shaker until the cover slip surfaces are clear.

5. Put the cover slips back on test tube rack. Pipette 0.5 % gluteraldehyde to cover the treated surface of the cover slips. Incubate for 30 minutes at room temperature in the hood. Ware gloves.

6. Collect the used glutaraldehyde in liquid waste. Wash as in step 4 and let air-dry. Activated cover slip may be stored in a dessicator for two weeks. Cover slips may be mounted onto chamber dishes before proceeding with the following steps.

7. Mix 5 ml of acrylamide solution in a small beaker according to the dilution scheme below. Beads are usually added at a volume of 50 ul. DO NOT ADD BEADS YET.

Final Acrylamide/Bis	40%Acrylamide	2%Bis	1M HEPES	H_20+Other	Young's Modulus
8%/0.1%	1000 ul	250 ul	50 ul	3700 ul	?? kN/m2
8/0.08	1000	200	50	3750	75
8/0.06	1000	150	50	3800	30
8/0.05	1000	125	50	3825	23
8/0.04	1000	100	50	3850	17
8/0.03	1000	75	50	3875	14
8/0.02	1000	50	50	3900	10
5/0.12	625	300	50	4025	33
5/0.10	625	250	50	4075	28
5/0.08	625	200	50	4125	24
5/0.06	625	150	50	4175	15
5/0.05	625	125	50	4200	??
5/0.025	625	63	50	4262	7
3/0.10	375	250	50	4325	??

8. Degas the solution for 20 minutes to remove oxygen, which inhibits acrylamide polymerization.

9. Sonicate the fluorescent beads for 1-2 minutes in a bath sonicator.

10. Add beads, 30 ul ammonium persulfate, 20 ul TEMED. Seal the beaker with parafilm and mix gently by swirling.

11. Pipette the acrylamide mixture onto the activated cover slip. Use 15 ul for a 75 um-thick gel. Quickly place a 22 mm circular cover slip onto the acrylamide droplet and invert the chamber dish.

12. Let acrylamide polymerize for 30 minutes.

13. Flood the surface with ~2 ml of 50 mM HEPES. Remove the circular cover slip with two pairs of fine tipped tweezers.

14. Rinse the substrate well with 50 mM HEPES. The substrate may be stored at 4°C for 2 weeks.

15. Remove as much liquid form the substrate as possible without drying, then layer 200 ul of the sulfo-SANPAH solution on top.

16. Place under 302 nm UV, at a distance of 2-3 inches from two 15W tubes, for 5-8 minutes. The solution will darken when activated.

17. Repeat steps 15 and 16

18. Wash with 50 mM HEPES to remove excess reagent. Do this quickly.

19. Add the protein to be coupled and incubate either 4 hours at room temperature or overnight in the cold room on a shaker.

20. Rinse with PBS and store coated substrates in the cold room for up to a week.

21. Before plating cells, expose the gel to UV for 15 minutes.

22. Replace PBS with complete culture medium. Place in incubator for 1 hour to allow equilibrium.

Notes

1. Young moduli were measured with a difficult, relatively unreliable bead indentation method. The values can be higher than those obtained with other methods. We have developed a more reliable method and will published updated values.

2. Bonding with coverslip is now performed with a simpler method using bind silane available through Sigma Aldrich.

References

Wang, Y.-L. and Pelham, R.J. Jr. (1998) Preparation of a flexible, porous polyacrylamide substrate for mechanical studies of cultured cells. *Methods Enzymol.* 298:489-496

Beningo, K.A., Lo, C.-M. and Wang, Y.-L. (2002) Flexible polyacrylamide substrata for the analysis of mechanical interactions at cell-substratum adhesions. *Methods Cell Biol.* 69:325-