**PAA Gels Between Coverslips**

**Materials**

40% acrylamide stock 2% bis-acrylamide stock

1M HEPES stock sulfo-SANPAH

10% ammonium persulfate solution TEMED

ECM protein (e.g. fibronectin) PBS

Methacrylate silanized coverslips sigmacote coverslips

epoxy

**Procedure**

1. Prepare solution of polyacrylamide of desired concentration (a total volume of 100uL per gel needed).

For a 10ml solution of 8% acrylamide and 0.3% bis-acrylamide:

2.0 ml of the 40% acrylamide stock solution

1.5 ml of the 2% bis-acrylamide stock solution

0.1 ml of the 1M HEPES stock solution

6.4 ml ddH2O

1. Add 1/200 volume of 10% ammonium persulfate (50 µl for 10 ml solution)
2. Add 1/2000 volume of TEMED (5µl for 10 ml solution)
3. Immediately pipette 50uL of PAA solution onto a 18mm (diameter) round methacrylate-silanized coverslip, and carefully place a second 18mm Sigmacote-treated coverslips plate on top of solution. Total volume can be optimized between 50-100uL.
4. Allow to polymerize for 30min.
5. After polymerization, carefully remove the Sigmacote coverslip and move the gels (attached to the bottom coverslip) to a 12-well plate. One gel per plate.
6. Prepare 50mM solution of sulfo-SANPAH.

For a 24ml solution (1mL in each well of 12-well plate):

Dilute 6mg SANPAH powder with 24mL 50mM HEPES (pH 8.5)

1. Add prepared solution of sulfo-SANPAH sufficiently to immediately cover gel surface – 1mL is sufficient for gels in a 12-well plate.
2. Expose gels to UV light (365nm bulb, 2.5 in. away) for 15min, this photoactivation will darken the chemical from an orange to a brown color. Every few minutes, move the lamp around so that all gels are getting ample UV exposure.
3. Rinse gels 4x5min in 50mM HEPES.
4. Repeat steps 8-10.
5. Add preferred ECM protein (e.g. fibronectin, collagen, laminin) to acrylamide in HEPES or PBS buffer. React overnight. For upside down reactions on parafilm, need 100uL of protein solution per gel. Total surface area of a 18mm round coverslip is 2.54cm2.
6. Rinse gels 4x5min in PBS.
7. Epoxy coverslip side of gel to new 12-well dish, add enough PBS to cover gels and keep hydrated, and UV sterilize in hood ~20min.
8. Add cells to wells in concentration best fitting type of study. <5K cells per gel in 12-well plate for migration studies.