PEG-PC Gel Protocol

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Methacrylate Silane Cover Slips

1. O2-plasma treat cover slips for 10 minutes.
2. Make up 100 mL of 95% ethanol. Adjust the pH to 5.0 with glacial acetic acid.
3. Measure out 98 mL of the pH 5.0 95% ethanol. Add 2 mL of 3-(Trimethoxysilyl)propyl methacrylate to bring it to 2% v/v.
4. Add the cover slips to the solution in a beaker and mix gently for 2 minutes.
5. Pour off the solution and rinse with 200 proof ethanol 3 times.
6. Dry the cover slips at 120C and atmospheric pressure for 15 minutes.
7. Store cover slips in a desiccator.

Sigmacote Cover Slips

1. Mix cover slips in Sigmacote for 20 minutes.
2. Pour off Sigmacote (into waste bottle), wash 3 times with 200 proof ethanol.
3. Dry at room temperature under vacuum for ~15 minutes.
4. Store cover slips in a desiccator.

PEG-PC Gels on Cover Slips

1. Make a solution of 20% w/v 2-Methacryloyloxyethyl phosphorylcholine (MPC) in PBS.
2. If it does not dissolve 100% (it won’t), centrifuge the solution at high G’s for 2 minutes.
3. Carefully pipette the clarified solution off the pellet. Be careful as the pellet is soft and easily disturbed.
4. Each cover slip requires 75 uL of polymer solution. Measure out the appropriate volume of 20% MPC.
5. Add the appropriate amount of PEGDMA. For example, if making 3% PEG and you measured out 300 uL of 20% MPC, add 3\*(1/100)\*300 uL = 9 uL of PEGDMA. Mix thoroughly. Degas the solution with ultrapure N2 for ~30 seconds.
6. Make fresh 20% w/v Irgacure 2959 in 70% ethanol. Degas with ultrapure N2 for ~5 minutes.
7. Add 40 uL of 20% Irgacure per 1 mL of polymer solution. Mix gently by inverting so as to not reintroduce dissolve oxygen into the solution.
8. Place 75 uL aliquots of polymer solution on the methacrylate silane cover slips. Carefully cover with a Sigmacote cover slip.
9. Treat with UV at 5-6” distance for 6 minutes.
10. Carefully remove the Sigmacote coverslip with fine forceps.
11. Store the gels in PBS over night.

Sulfo-SANPAH and Protein Coupling

1. Make a solution of 0.2 mg/mL Sulfo-SANPAH in 50 mM HEPES pH 8.5.
2. Place gels in 12 well plates. Add 1 mL of Sulfo-SANPAH solution to each well.
3. Treat with UV for 15 minutes.
4. Remove the solution and wash 2x with HEPES buffer.
5. Add another 1 mL of Sulfo-SANPAH solution to each well. Treat with UV for an additional 15 minutes.
6. Wash each gel 3x with PBS.
7. Immediately place them gel-side down on 100 uL droplets of protein solution in a ‘wet box’ and incubate over night.
8. The next day, wash the gels thoroughly with PBS and proceed with blocking, sterilization and experiments.