**COMPREHENSIVE PEG-PC GEL PROTOCOL**

**Last updated 2/4/2013 by W.H.**

**MATERIALS**

2-Methacryloyloxyethyl phosphorylcholine (Sigma)

Poly(ethylene) glycol dimethacrylate, MN ~ 750 (Sigma)

PBS

0.22 µm PES syringe filters

Luer-lock syringes

microcentrifuge tubes

vortexer

*For UV curing*: Irgacure 2959 (Ciba), a high-intensity UV light source, methacrylate silane-treated coverslips and Sigmacote-treated coverslips

*For thermal curing*: ammonium persulfate and TEMED (Bio-Rad), vacuum oven/chamber, N2 gas, methacrylate silane-treated glass-bottomed 96-well plates

**PREPARATION OF PRE-POLYMER SOLUTION**

1. Make a solution of 20% (w/v) MPC in PBS i.e. 200 mg MPC per mL of PBS (actual w/v after volume change is ~17%) and vortex to dissolve. Calculate the volume to make on a basis of 75 µL pre-polymer solution per coverslip or 40 µl/well in a 96-well plate.
2. Evenly distribute 20% MPC solution into microcentrifuge tubes for each PEG-PC concentration to be made.
3. Add the appropriate amount of PEGDMA. For example, if making 3% PEG-PC and you measured out 300 µL of 20% MPC, add 3% of that volume as PEGDMA i.e. 0.03 \* 300 µL = 9 µL of PEGDMA. Mix thoroughly by vortexing. (**N.B.:** If making PEG-PC over 10%, the % of MPC and PEGDMA is calculated differently because the volume changes are substantial).
4. If gels are to be used for cell culture experiments, syringe filter each solution.
5. Degas each solution with ultrapure (grade 5) N2 for ~30 seconds (this is especially important for thermal curing). Be sure to sterilize the degassing needle with 70% ethanol to keep the solutions clean, and run ethanol through the needle when finished so it does not get clogged.

**POLYMERIZATION ON COVERSLIPS**

1. Make *fresh* 20% w/v Irgacure 2959 in 70% ethanol, vortex to dissolve (it takes a lot of vortexing). Degassing this solution is not usually necessary, but it cannot hurt.
2. Add 40 µL of 20% Irgacure per 1 mL of polymer solution. Mix gently, by inverting or pipetting, so as to not reintroduce dissolve oxygen into the solution.
3. Place 75 µL aliquots of pre-polymer solution on methacrylate silane-treated cover slips. Carefully cover with a Sigmacote-treated cover slip.
4. Treat with UV at 5-6” distance for 7 minutes.
5. Carefully remove the Sigmacote coverslip with fine forceps.
6. Store the gels in PBS overnight.

**POLYERMZATION IN 96-WELL PLATES**

1. Make *fresh* 20% ammonium persulfate (APS) in deionized water, vortex to dissolve. Degassing this solution is not usually necessary, but it cannot hurt.
2. To polymerize, add 20% APS to pre-polymer solutions at a 1:80 ratio, and add TEMED 1:800. i.e. 1000 µL pre-polymer / 80 = 12.5 µL 20% APS and (1000/800) = 1.25 µL TEMED. **N.B.:** After addition of APS and TEMED it is possible for the solution to polymerize on the bench top, so it is recommended that each PEG-PC concentration be done separately i.e. don’t add APS and TEMED to every pre-polymer solution before aliquoting into wells.
3. After addition of APS and TEMED, aliquot 40 µl/well into the wells of a methacrylate silane-treated 96-well plate.
4. Repeat for every PEG-PC concentration being made.
5. Place 96-well plate in vacuum oven at room temperature with the cover off and flow ultrapure (grade 5) N2 at 25 psi through the chamber for 5 minutes.
6. After 5 minutes of flowing N2, close the inlet and outlet to the vacuum oven tightly and close the N2 valve.
7. After 5 additional minutes, gels should have formed.
8. Swell in PBS overnight. Use 100 µl/well, except for 0.5% PEG-PC gels use 200 µl/well (because they swell so much).