**PEG-MAL Hydrogel Protocol**

Materials:

4-arm Polyethylene Glycol-Maleimide (PEG-MAL), MW 20 kDa, >95% purity (JenKem)

Cross-linking polymer

* Pan-MMP (degradable) sequence: GCRDQGWI↓GQPGDRCG (genscript)
	+ Note: Other degradable sequences can be made for specific MMPs
	+ J, Patterson, JA Hubbell, Biomaterials 31 (2010) p.7836-7845
	+ Generally, these peptides are purchased in aliquots of 0.5mg to avoid weighing errors
* Linear PEG dithiol, MW 1.5kDa or 1kDa, >95% purity (JenKem or Sigma)

Adhesive ligand –

* RGD sequence: RGDC, >95% purity (GenScript)
* 200mM solution in PBS @ pH 7.4
* Note: other adhesive ligands can be added to the gel

PBS, pH 7.4

2 mM Triethanolamine (TEOA) in PBS, pH 7.4

Gel making protocol:

1. Gels are calculated by weight percent. Crosslinking is determined by matching moles of reactive ends of crosslinker to moles of reactive ends of 4 arm PEG-MAL. (Example calculations are below)
2. Warm polymer solutions to room temperature and weigh out appropriate mass of polymers needed.
3. Make 2 mM TEA in PBS, pH 7.4 (Maleimide-thiol reaction occurs at pH 7-7.5)
4. Dissolve crosslinking polymers in sterile TEOA solution and PEG-MAL in PBS or serum free medium
5. Functionalize hydrogel
	1. Mix PEG-MAL solution and RGD solution at 100:1 ratio for a final solution of 2mM RGD in the hydrogel
	2. React at room temperature for a minimum of 10 min
6. If encapsulating cells, begin to split the cells and spin down the desired cell amount into a pellet. Range between 5,000 to 40,000 cells per gel.
7. Re-suspend cell pellet in the PEG-RGD solution. Minimize the time cells suspended in un-crosslinked PEG solution. If you are making multiple plates, stage them by putting into the incubator. The goal is to have cells out for no longer than 10 minutes.
8. Cast hydrogels by mixing PEG-RGD-cells with crosslinking solution
	1. We normally do this at a 10:1 ratio PEG-MAL:PDT. The PDT solution is first placed on the bottom of the plate and then mixed with the PEG-MAL solution
	2. Gels polymerize very quickly (~30secs) see below for tips to regulate the reaction
	3. For cell culture we use a 10 uL to maximize oxygen diffusion into the hydrogel
	4. We normally use a 20K PEG MAL, but the reaction conditions and volumes for the reaction change based on the arm length of the polymer you are using.
9. Incubate gel at 37C for 10minutes (max 20 minutes)
10. Add media (or PBS if no cells) to swell gel (minimum 18 hrs)

Notes:

TEOA

* Higher TEOA concentrations may be used to decrease reaction time and increase reaction rate.
* Maleimide-thiol reaction occurs at pH 7-7.5. pH to 7.4 when encapsulating cells
* Gels will not gel at pH of 5.5

PEG-MAL

* Should be stored with nitrogen gas at -20C in a sealed container
* A 0.45 um spin filter can be used to sterilize polymers, but a 20 kDa PEG-MAL will not fit through it, so it can be sterilized in EtOH.

Peptides

* Should be stored at -20C.
* Each adhesive ligand will affect the gelation process differently and must be experimented with. Check the pH of the solution if you have gelation problems.

Example: To make a 10wt% gel

 Select $100\frac{mg}{ml}$ or of PEG-MAL

$$100\frac{mg}{ml}\*\frac{1g}{1000mg}\*\frac{1 mol}{20000 g PEG-MAL}=5x10^{-6}\frac{mol}{ml}$$

 Since PEG-MAL has 4 ends, we need to multiply for 4:

$$5x10^{-6}\frac{mol}{ml}\*4 rxn ends=20x10^{-6}\frac{mol}{ml}$$

 and crosslinker has 2 ends, we need to divide by 2 (and multiply by 10\*):

$$20x10^{-6}\frac{mol}{ml}\*\frac{10}{2 rxn ends}=100x10^{-4}\frac{mol}{ml}$$

 We need $100x10^{-4}\frac{mol}{ml}$ of PEG dithiol or $0.15\frac{g}{ml}(\frac{mg}{ul})$

$$100x10^{-4}\frac{mol}{ml}\*\frac{1500 g}{mol}=0.15\frac{g}{ml}(\frac{mg}{ul})$$

For a 7.5 wt % we need $0.1125\frac{g}{ml}(\frac{mg}{ul})$

For a 15 wt % we need $0.225\frac{g}{ml}(\frac{mg}{ul})$

For a 20 wt % we need $0.30\frac{g}{ml}(\frac{mg}{ul})$

\*Note: We normally add 1 uL or PDT to 9uL of PEG-MAL for a total of a 10uL gel. The 10x factor is to account for the volume changes in the solutions. You can change the volume and concentrations you use to make the gels, but we found this works best with a 20K PEG.