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Proteins/peptide functionalization of PEG-PC gels on coverslips or 96-well plates

Materials and solutions:

- Protein/Peptide solution in Sterile PBS, pH 7.4  
(Concentrations between 1-10  $\mu\text{g}/\text{cm}^2$  have been used in the lab)

The example below is a description for one 18 mm coverslip and 1  $\mu\text{g}/\text{cm}^2$ :

$$\begin{aligned} \text{Area} &= \pi r^2 \\ \text{Area} &= \pi(0.9 \text{ cm})^2 = 2.54 \text{ cm}^2 \end{aligned}$$

Mass need per one coverslip:

$$1 \frac{\mu\text{g}}{\text{cm}^2} * 2.54 \text{ cm}^2 = 2.54 \mu\text{g}$$

Volume needed of protein/peptide per one coverslip:

Note: You will need 95  $\mu\text{L}$  (18 mm) or 70  $\mu\text{L}$  (15 mm) droplets of protein/peptide solution

Protein	Stock concentration	Volume needed of protein per one coverslip
Collagen	3 $\mu\text{g}/\mu\text{l}$	$2.54 \mu\text{g} / 3 \mu\text{g}/\mu\text{l} = 0.846 \mu\text{l}$
Laminin	1.19 $\mu\text{g}/\mu\text{l}$	$2.54 \mu\text{g} / 1.19 \mu\text{g}/\mu\text{l} = 2.13 \mu\text{l}$
Fibronectin	1 $\mu\text{g}/\mu\text{l}$	$2.54 \mu\text{g} / 1 \mu\text{g}/\mu\text{l} = 2.54 \mu\text{l}$

Complete volume with PBS (ex: for laminin)  $\rightarrow 2.13 \mu\text{l} + 92.87 \mu\text{l} = 95 \mu\text{L}$

Note: For collagen I, concentrations between 1-5  $\mu\text{g}/\text{cm}^2$  use sterile PBS, pH 3.8

- Sulfo-SANPAH (ProteoChem, Catalog# c1111)
- HEPES buffer: 50 mM HEPES, pH 8.5.
- Tweezers
- 12-well plate/96-well plate
- Epoxy (If time-lapse microscopy will be used)
- Petri dish (non-cell culture)

Procedure:

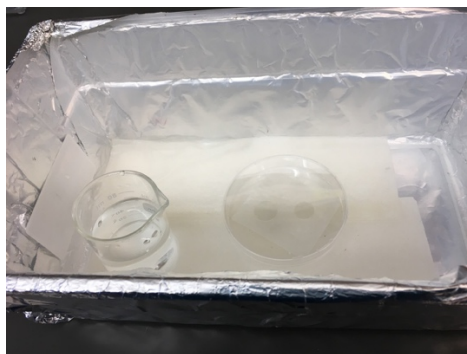
Note: Functionalize peptides/proteins to PEG-PC gels on coverslips or 96-well plates (For PEG-PC gels on coverslips or 96-well plates, refer to protocol: PEG-PC Gels on Coverslips or 96-well plate)

1. Prepare 0.3 mg/ml (for coverslips) or 0.6 mg/ml (96-well plate) solution of sulfo-SANPAH in HEPES buffer (keep solution in the dark).  
Example: For 20 ml of 0.6 mg/ml solution of sulfo-SANPAH in HEPES buffer

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- Prepare a 0.1 mg/ $\mu$ L sulfo-SANPAH solution: Dilute 100 mg sulfo-SANPAH powder with 1000  $\mu$ L DMSO (You can prepare a big batch and store in the -80°C freezer)
  - Pipet 120  $\mu$ L sulfo-SANPAH solution into 20 ml of HEPES buffer
2. For coverslips, place coverslips gel side up in a 12-well plate. For a 96-well plate, gel are already confined into the plate from protocol PEG-PC Gels on Coverslips or 96-well plate (no action needed at this step for 96-well plate).
  3. Add prepared solution of sulfo-SANPAH sufficiently to barely cover gel surface: 1 ml per well for a 12-well plate (for coverslips), or 75  $\mu$ L per well (for a 96-well plate).
  4. Expose treated surface of gels to UV light for 10-30 min, this photoactivation will darken the chemical considerably. Be sure to have the color changed.
  5. Rinse surface 3X with HEPES buffer, to remove excess reagent (1 ml per well for a 12-well plate (for coverslips), or 75  $\mu$ L per well (for a 96-well plate)).
  6. For gels on coverslips: Use a container large enough to hold coverslips in petri dishes, add water to soak paper towels and place a beaker with water inside the container (see picture below). Add 95  $\mu$ L (18 mm) or 70  $\mu$ L (15 mm) droplets of protein/peptide solution on parafilm inside petri dish. Carefully use tweezers to place a gel on each protein/peptide solution droplet. React at room temperature for at least 3 h.



7. For gels on 96-well plate: Add 75  $\mu$ L per well of protein/peptide solution in PBS to the gel. React at room temperature for at least 3 h.
8. For gels on coverslips: Move the coverslips back into the 12-well plate, flipping the orientation (protein/peptide side up) Wash gels (1 ml per well) thoroughly with PBS buffer (2X). Epoxy the coverslips to the well plate if you will be doing time lapse microscopy.
9. For gels on 96-well plate: Wash gels (75  $\mu$ L per well) thoroughly with PBS buffer (2X)
10. Optional: Sterilize gels with UV for 1 h.
11. Seed cells.