SOP TITLE:	VERSION:	
PM-PD-SOP2.1 Coating well-plates with PeptiMatrix hydrogels	1.2	
AUTHOR & RESEARCH TEAM:	STATUS:	
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1. BACKGROUND

Self-assembling peptide hydrogels can be used in a variety of cell culture applications, including providing a 2.5D environment where cells are seeded on a coated well-plate. PeptiMatrix™ hydrogels are formed from short peptide sequences of between 8-20 amino acids. At the correct pH and temperature, they self-assemble to form a stable entangled network of nanofibers. The non-covalent nature of the peptide interactions enables the hydrogel to undergo gel-liquid-gel transitions upon shear stress, allowing to create an uniform coating layer.

2. RISK ASSESSMENT

Please refer to PM-RA0 and PM-RA2 for a full risk assessment of the following protocol. Ensure that you fully understand the potential risks and have received the appropriate training before starting any work.

3. MATERIALS

- PeptiMatrix Core (PMCORE125)
- Class II Microbiology Safety Cabinet (or appropriate class for your cells/materials)
- Centrifuge
- Centrifuge tubes (e.g., 15 mL falcon tube)
- 80 °C oven
- P1000, P200, P20, P10 filter tips
- P1000, P200, P20, P10 pipette
- 24-, 96-, or 384-well plate **OR** 24-well plate with 24-well cell culture inserts (for indirect co-culture experiments)
- Cells to be seeded
- Cell culture media appropriate for your cells
- DPBS, no calcium, no magnesium (e.g., Gibco 14190144) (optional)
- Soluble matrix additions (e.g., recombinant laminin) (optional)

4. METHODS

Final gelation / Cell seeding

The thickness and viscosity of the coating can influence cell behaviour, hence the optimal conditions are dependent on the experimental plan and intended outcome. This protocol outlines how to coat each well of a 96-well plate with a 1 mm thick layer as an example. Scale up or down according to your requirements.

1. Transfer 1 mL of hydrogel to a 15 mL centrifuge tube and centrifuge at 600 x g for 1 min to remove any air bubbles.
   a. **OPTIONAL:** If gels have been stored for longer periods (>1 month), it can be beneficial to heat gels in an 80 °C oven for at least 20 min before prior to final gelation to ensure a homogeneous end gel. Gels of lower concentration will be free-flowing at this temperature. Gels of higher concentration may remain self-supporting but will be less viscous.
   b. This can be done either before or after transferring the 1 mL of hydrogel to a 15 mL centrifuge tube (the vials and caps are heat resistant). The advantage of doing this before transfer is that it can make it make the transfer easier, as the gels will be less viscous at higher temperatures.
   c. **CAUTION:** Ensure proper temperature control during this step, as temperatures above 90 °C can damage the gels.
2. Transfer the gel to the 37 °C water bath. The gels should be self-supporting again when cooled to this temperature.
3. **OPTIONAL:** The gel can now be diluted to adjust the end viscosity, depending on culture requirements. We recommend diluting with DPBS to preserve the salt balance of the gels. Typical dilution ratios are 25:75, 50:50, or 75:25 DPBS:gel. To dilute the gels, we recommend combining the required volumes of gel and DPBS in a centrifuge tube and performing a brief vortex and centrifuge step.
4. **OPTIONAL:** If you would like to add soluble matrix components to the gel environment, these can also be added at this stage. Prepare your soluble matrix components (e.g., recombinant laminin) per the manufacturer's recommendation and then mix this into the gel by a combined action of stirring and reverse pipetting.* The ideal type and concentration of optional matrix additions will depend on the cell type and application.
5. For a 1 mm thick layer, add 50 µL of gel to each well of the 96-well plate.
6. Gently swirl the plate and then centrifuge for 1 minute at 600 x g for 1 minute to ensure an even surface.
7. Carefully add 200 µL of cell culture medium to cover the surface of the gel coating. Add the culture medium by pipetting slowly on top of the gel to avoid disturbance of the coating.
8. Incubate at 37°C for 30 minutes to allow for final gelation. Thicker coatings may require longer incubation times (1 h).
9. During incubation, prepare your cell suspension for seeding. A good initial seeding density is between 10,000 and 40,000 cells per well of a 96-well plate, but may need to be optimised based on cell type and anticipated length of experiment.
10. Remove the cell culture medium from the top of the gel.
11. Use the plate immediately by adding your cell suspension to the top of the gel and returning to the incubator.
12. Change media the next day and then as needed during culture, depending on cell type and number seeded.
    a. **TIP:** When performing media changes during culture, it is also helpful to exchange only 75% of the media each time (e.g., exchanging 150 µL of media instead of 200 µL when using a 96-well plate), to avoid disturbing the gel.

* see section below for instructions on how to reverse pipette

Notes on Gel pH

1. The precursor hydrogels (prior to final gelation) have a slightly alkaline pH, such that complete gelation does not occur until after combining with cell culture media.
2. After combining with cell culture media, the gel is rapidly neutralised to pH 9 and continues to drop to 7 over the next 12 hours.
3. If your cells are particularly sensitive to alkaline pH, you can perform 2x 30 min media washes on the gels after plating, which will more rapidly neutralise the gels to pH 7 before seeding your cells.
   a. Typically, we do this with more sensitive stem cell and primary lines, but is not necessary with continuous cancer cell lines

5. DISPOSAL

Hydrogels seeded with cells and/or matrix components should be treated in the same way as suspensions of the cell type and matrix components in use.

6. REVERSE PIPETTING

Forward pipetting is considered a standard technique and is ideal for most aqueous solutions. In forward pipetting, the target volume is aspirated and dispensed, and a separate blowout step is used to completely empty the tip by pressing the plunger to the second stop.

In reverse pipetting technique, the pipette aspirates the selected volume plus an excess volume. After dispensing, the excess volume remains in the tip and is then discarded. This technique is recommended when handling more viscous samples (such as hydrogels).

How to reverse pipette:

1. Adjust volume of pipette.
2. Fit the tip into the pipette tip cone.
3. Press the operating button all the way to the second stop.
4. Insert the tip just under the surface of the liquid.
5. Slowly release the operating button, allowing it to return to the starting position. For particularly viscous liquids, you may need to wait a few seconds for the full desired volume to be drawn into the tip.
6. Carefully withdraw the tip from the liquid.
7. Press the operating button smoothly to the first stop to deliver the desired volume. Wait one second.
8. Discard the remaining liquid by pressing the operating button to the second stop.

To see a video of this protocol, see: Tutorial: How to Reverse Pipette? (youtube.com)

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