Manchester BIOGEL Protocol

How to perform 2D cell culture

This protocol describes pre-conditioning of PeptiGels® using culture media and seeding cells on the surface of the pre-conditioned hydrogels. This protocol is designed for seeding cells on the surface of a 0.2 mL gel inside a 12-well insert (Greiner-Bio-One) and changing PeptiGel® volume is necessary when culture container is changed, e.g. 0.35 mL hydrogel for a well in 48-well plate.

This protocol also describes the use of a LIVE/DEAD™ assay (L3224, ThermoFisher Scientific) coupled with fluorescence imaging to assess viability of cells *in-situ* on the surface of the hydrogel.

Cell culture

1. Centrifuge the PeptiGel® inside a falcon tube at 3000 rpm for 1 minute to remove air bubbles.
2. Pipette 0.2 mL of PeptiGel® using a positive pipette, or a micro-pipette with a cut-end tip (cut with sterile scissors), and place the hydrogel onto the bottom surface of a insert, ensure the gel to cover the entire insert bottom surface (Note: centrifuging the insert in a 12-well plate for 1 minute at 3000 rpm or tapping the insert against a sterile surface for 20 times is useful to get a flat hydrogel surface).
3. Add 1 mL cell culture media in the well containing the insert, incubate the gel with culture media for 30 minutes in a 37℃/ 5% CO_2 incubator.
4. Add another 0.2 mL culture media on the surface of the PeptiGel® in the insert and incubate for overnight.
5. On the next day (cell seeding day), warm up culture media and other relevant cell culture reagents (e.g. trypsin) to room temperature or 37 °C.
6. Trypsinize cells and perform cell counting. Then, prepare a cell suspension according to the final required cell seeding density (e.g. for 50000 cell/cm^2 of hydrogel surface, with the gel having a surface area of 1.13 cm^2 in a 12-well insert, 56500 cells/insert is needed. Prepare a 2.8 million cells/mL cell suspension and add 200 µL in each insert).
7. Using a micropipette, remove the culture media used to pre-condition the hydrogel, from both on the surface of the gel and in the well containing the insert.
8. Pipette 0.2 mL of the prepared cell suspension into each insert containing a gel, and pipette 1.0 mL of fresh culture media in each well containing the inserts.
9. The following day, change the culture media and then every other day (in general).

Viability assessment

1. Warm the LIVE/DEAD™ reagents (Calcein AM and Ethidium homodimer-1) to room temperature.
2. Pipette 20 µL of Calcein-AM into 10 mL of phosphate buffered saline (PBS), vortex to ensure homogeneity.
3. Pipette 5 µL of Ethidium homodimer-1 (EthD-1) into the above solution, and further vortex to obtain a homogenous solution.
4. Remove the culture media from the 24-well inserts or 48-well plates, including any culture media underneath the well plate.

5. Place 300 µL of the assay solution into the insert/well containing the PeptiGel®-cell constructs and incubate for 1–2 hours, depending on volume of the hydrogel.
6. Place the PeptiGel® onto a glass slide, and cover with a coverslip. Image the cells using a fluorescence microscope at an excitation wavelength of 495 nm and two emission wavelengths; 515 and 635 nm, respectively.
   Note: gentle pressure can be applied to flatten the hydrogel and aid imaging.
   Note: for further information on the cell distribution within the hydrogel, confocal microscopy can be used. With this technique, the application of any pressure is not recommended.

If you have any questions or queries when working with PeptiGels®, please contact our technical support team on +44 (0) 1625 238 800 or email info@manchesterbiogel.com.