3D culture protocol

Introduction

3D culture for lung cancer cells, adapt from mammary epithilial cell 3D culture protocol
http://muthuswamylab.cshl.edu/protocols/3D%20morphogenesis.pdf

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Materials

- Growth Media: DMEM+10%FBS+Pen Strep
- Growth factor-reduced Matrigel (BD, Cat# 354230)
- Collagen I from Rat tail (BD, Cat# 354236)
- Sterile 1xPBS and 10xPBS
- 1N NaOH
- 1x Trypsin:EDTA
- 8-well or 4-well chamber slides (Corning 354108)

Procedure

Coat the Slides with Matrigel

1. Thaw Matrigel on ice (Allow 1-2 hrs for small aliquots (<2ml) and 4-6 hours for larger aliquots (3-10ml)). Note: Always keep Matrigel on ice; it will solidify at room temperature. Pre-chill all the pipet tips.

2. Make Collagen I (0.5mg/ml): Matrigel mix (1:1)

   Collagen I (3.83mg/ml) 780ul
   H2O 1.9ml
   10xPBS 300ul
   /Total 3ml, mix well
   Add 3ml Matrigel, mix well
   add 40ul 1N NaOH, mix well using pipet
   Use pipet to mix to avoid making air bubbles

3. CRITICAL Add 50 µl Matrigel to each well of an 8 well chamber slide. Spread Matrigel evenly to the edge of the wells using a p10 tip, taking care not to generate a meniscus or air bubbles. You may want to perform this step on ice until you are comfortable with the procedure. At this step, the well is not evenly coated with Matrigel.

4. Place chamber slides in cell culture incubator and allow 5 minutes for the Matrigel to partially solidify. Add another 50ul Matrigel to each well, Spread Matrigel evenly with a p10 tip, taking care not to generate a meniscus or air bubbles. The surface of the Matrigel should be even at this point.

5. Place chamber slides in cell culture incubator and allow 20 minutes for the Matrigel to solidify.

Plate the cells

6. Trypsinize cells (check under the scope to make sure the cells are trypsinzed into single cells) and resupend them in 1 ml media. Add 2 more ml of resuspension media and wait 2 minutes. This step ensures the deactivation of trypsin.

7. Spin cells at 1000RPM for 3 minutes. Resuspend cells in 10 ml GM.

8. Count cells and aliquot 1x10E+5 cells into a fresh 15 ml conical tube containing 4ml of GM. This will generate2,500 cells/100µl --Tube A

9. Prepare GM containing 10% Matrigel:Matrigel mixture. Use media that has been cooled on ice to avoid solidification of Matrigel --Tube B

10. Mix Tube A and Tube B 1:1 and plate 400 µl of the Matrigel:cell mixture into 1 well of the Matrigel coated chamber slide prepared in step 3. This generates 5000 cells/well.

11. Gently transfer the chamber slide into the cell culture incubator.

12. Change media every 4 days with GM.