Primary BPH stromal cell culture and prostatic epithelial 3D culture

1. Primary prostate tissue specimens obtained from surgeries are stored in RPMI 1640 medium at 4 °C (recipe below).
2. Wash prostate tissue specimens with 3 ml ice cold PBS 3 times.
3. Mince tissues (into ~2 mm pieces) in a sterile 6 cm dish using sterile scissors. Transfer up to 10 pieces using sterile tweezers into a 1.5 ml Eppendorf tube.
4. Minced tissue specimens (1-2 mg) are digested by 1 hr incubation at 37 °C in 1 ml of 2.4 U/ml Dispase II (Cat # 04942078001, Roche Applied Science, Indianapolis, IN) in RPMI-1640 media on a rocker.
5. Centrifuge minced tissue suspension at 180 x g for 5 mins.
6. Aspirate the supernatant and resuspend cell pellet in 1 ml of 50/50 DMEM/F12 (recipe below).
7. Add 4 ml 50/50 DMEM/F12 to a 6 cm culture dish. Add 1 ml of cell suspension with gentle mixing via repeated pipetting. Total volume is 5 ml.
8. Incubate plated cells at 37 °C, 5% CO_2 for 5 days without disturbing. Add 2 ml of fresh medium on top of medium to avoid evaporation on the 5^{th} day. Stromal cell proliferation should be evident by day 7.
9. When stromal cells reach 95% confluence, cells are serially passaged using trypsin:EDTA (0.25%:0.53 mM) solution, then neutralized with 50/50 DMEM/F12. Spin down cells and remove the supernatant from the pellet. Cells are then resuspended with 50/50 DMEM/F12 and split 1:3. Aliquots can be stored at -80 °C as passage 1 (freezing medium is 10% DMSO in 50/50 DMEM/F12) or reserved for subsequent experiments.
10. Primary cells are used in experiments from passage 1-15.

Stromal conditioned medium

1. Seed primary stromal cells (200,000 cells) into 6 cm culture dishes with 5 ml of 50/50 DMEM/F12 and culture to 100% confluence (~ 2 days).
2. Collect conditioned medium (CM) by aspirating from the culture dish into a 15 ml falcon tube and replace with fresh medium (5 ml).
3. Centrifuge the stromal CM at 180 x g for 5 min to remove cellular debris and collect the supernatant. Store the CM at -20 °C.

3D Matrigel culture of prostatic epithelial cells

1. Seed prostate epithelial cells (300,000 cells) (i.e., BPH-1 cultured in RPMI 1640 or BHPrE1 cultured in 50/50 DMEM/F12) into 6 cm plates until 80% confluent.
2. Warm a sterile 24-well plate at 37 °C for 15 mins.
3. Trypsinize epithelial cells with trypsin:EDTA (0.25%:0.53 mM) and neutralize with 50/50 DMEM/F12. Spin down cells and remove the supernatant from the cell pellet. Add 5 ml of DMEM/F12 and determine the number of cells. Based on the total number of cells to be used in 3D culture (i.e., 2,520 cells/well), transfer the proper volume of the cell suspension to a sterile tube. Spin down the cells and remove the supernatant from the pellet. Resuspend cells in Matrigel at a concentration of 63 cells/1 µl of Matrigel. Avoid generating bubbles in the Matrigel.
4. Carefully seed 40 µl of cell:Matrigel suspension in the center of each well in the 24-well plate.
5. Cover the plate and place it upside down in a 37 °C incubator for 15 mins to allow cell:Matrigel suspension to solidify.

6. Carefully add 500 µl stromal CM (see above) to each well. Add the CM along the edge of the well wall to avoid disturbing the Matrigel. Then, add an additional 500 µl fresh medium to each well. Culture the epithelial cells without disturbing for 2 days at 37 °C.
7. Remove media from plate and replace with a fresh mixture of 500 µl CM and 500 µl medium every 2 days.

RPMI 1640 medium

RPMI 1640 medium
1% L- Glutamine
1% Penicillin-Streptomycin
10% FBS

50/50 DMEM/F12

50/50 Dulbecco's modified Eagles medium (DMEM)/F12
1 µg/ml insulin-transferrin-selenium-X
0.4% bovine pituitary extract
3 ng/ml epidermal growth factor
1% L- Glutamine
1% Penicillin-Streptomycin
5% FBS