Human Mammary Epithelial Cells (HMEC)

From: Duke/UNC/UTA/EBI ENCODE group
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1) Source of cells: Primary mammary epithelial cells are obtained by Dr. Jeffrrey Marks under Duke IRB Protocol EIRB#12025. Cells are isolated from a breast reduction surgery at Duke University were isolated and cultured according to previously published protocols (see below for details of isolation and dissociation)

2) Lineage of cells: Primary

3) Donor information: Female age unknown

4) Karyotope: Assumption is that they are normal cells since they are primary but karyotyping has not been done on these cells.

5) Media for cell lines:

Mammary Epithieal Basal Medium (MEBM from Lonza CC-3151)
Growth Medium
500 ml MEBM (HEPES buffered)
2.5 µg EGF (part of bullet kit)
0.25 mg hydrocortisone (part of bullet kit)
2.5 mg insulin (part of bullet kit)
35 mg BPE (part of bullet kit)
2.5 mg transferrin (250 µl of a 10 mg/ml solution)
10 µM isoproterenol (1 ml of a 5 mM stock)

6) Growth conditions. These cells are grown at 3% CO2. Cells were grown in cell culture plates. The media is changed every two days. The media has almost no serum so when trypsinized, serum is added to quench the trypsin. Then the serum is washed off the cells and they are replated in MEBM growth media.

7) Cell passages: The cells are grown from the organoid stage which we call passage 0. The are grown to passage 3-4 to achieve a required density

Protocol for isolation, dissocitation, and growth of human mammary epithelial cells from Stampfer, M.R. & Yaswen, P., Culture systems for study of human mammary epithelial cell proliferation, differentiation and transformation. Cancer Surv. **18**, 7-34 (1993).

Dissociation Buffers:
Tissue Mix Medium: MEGM media without additives from Cambrex or Ham's F12 + insulin (10 ug.ml), penicillin (100 U/ml); streptomycin (100 ug/ml); polymixin B (50 U/ml) and Fungizone (3 ug/ml), store at 4C

Enzyme solution: Dissove collagenase (1500 U/ml) in appropriate amount of tissue mix medium at 37 C. Filter with prefilter attached through a 500 ml bottle top filter.Dissove hyaluronidase (1000 U/ml) in

appropriate amount of tissue mix medium at 37 C. Filter through the previous bottle top filter, trying to add the same volume of collagenase mixture and hyaluronidase mixture. Aliquot 30 ml vol into 50 ml conical tubes, and store at -70C up to one year. (This is a 5X solution).

1. Obtain human mammary tissue as discard material from surgical procedures.
2. Place material in sterile containers containing sterile buffer or tissue mix medium plus 10% FBS up to 72 h without significant loss of epithelial cell viability. If obtaining tissue from the Marks lab, the tissue should arrive in the tissue mix medium already, in a sterile bottle.
3. Separate the epithelial areas from the stromal matrix in sterile 150 mm Petri dishes using a combination of sterile scalpel, forcep and scissor. (epithelial areas appear as white strands embedded in he stromal matrix.) Dissect out these areas, scraping out the grossly fatty material.
4. Prepare 50 ml conicals for the tissue by adding 5 ml of FBS and 18.3 ml of Tissue Mix media.
5. 5. Transfer the minced epithelial-containing tissue into a conical centrifuge tube with tissue comprising no grwaeer than a third of the volume of the tube (16.7 ml), up to the 40 ml line on the tube. Bring the tube up to full volume with enzyme solution (10 ml) leaving only a small air space to allow for gentle mixing during rotation.

Digestion:

6. Place tubes on a tube rotator and rotate overnight at 37C.
7. Centrifuge tubes at 600 g for 5 min. Discard the supernatant fat and medium. Dilute a small aliquot of the pellet in medium to check in the microscope for degree of digestion. Digestion is complete when microscopic examination shows clumps of cells (organoids) with ductal, alveolar, or ductal-alveolar structures free from attached stroma.
8. If the tissue is not full digested, resuspend the pellet in fresh tissue digestion medium at approximately the same ratio of pellet to medium. Reincubate with rotation at 37C for another 4 to 12 hrs. Recentrifuge tubes and recheck pellet. If digestion is still not complete add fresh digestion medium and incubate again overnight. The concentration of enzymes in the digestion medium can be varied according to how much more digestion is required.

Washing and Freezing Buffers:
CPMI: Add 15 ml FBS, 10 ml DMSO to 75 ml of a 1:1 mixture of DME and F12 (H/H); shake gently and store indefinitely at -20C

9. Wash the remaining organoids and single cells with medium three times.
10. To enrich the preparation for ductal and lobular emelents and to eliminate the majority of free blood cells, fibroblasts and endothelial cells, three 30 min to 1 hr sedimentation steps at 1 g (on the bench top) should be employed.
11. Remove the supernatant of the stromal cells cultures.
12. A loss pellet of 5-10 ml of organoids should be obtained from each reduction mammoplasty preparation.
13. Centrifuge the organoids down at 600 g for 5 min, remove supernatant.
14. Add 1 ml CPMI for every 0.1 ml pellet.
15. Seed a test dish for each tube by placing 0.1 ml of resuspended material into 35-mmm dishes drop by drop to fill in different areas of the dish. Disperse the organoids on dish by gently knocking the dish sideways to spread out the medium. Let sit approximately 1 min, then add 1 ml of growth medium to dish. Incubate at 37 C and check for attachment and sterility the following day.
16. Store fractions in liquid nitrogen until use.

Thawing organoids:

17. Organoids should be thawed according to the procedures from the Marks lab.
18. Quickly thaw the cultures in a 37 C water bath. Place thawed organoids, drip by drip onto the surface of the 6 T-25 flasks with a Pasteur pipette to yield an even distribution. Add 2 ml of media to the other side of the flask. Slowly tip the flask over to have the medium cover the organoids without dislodging them.
19. After 1d of incubation at 37C, check that the organoids are attached. ADdd an addition 2 ml of fresh medium. Cell outgrowth should be visible by 24 to 48 h after seeding.
20. Cultures should be fed every 48 h or 3 times a week.
21. If fibroblastic cell growth is observed, remove by differential trypsinization.
22. Primary cultures should not be permitted to remain at confluence, or growth potential upon subculture will be diminished.