Passing Cells

Description

When cells are confluent, we pass them from one dish to three dishes, to synchronize the cell growth cycle and prepare for experiment.

Materials

1. PBS
2. Trypsin (in fridge), 1x for smooth muscle cells and 0.5x for endothelial cells (warm up in water bath)
3. DMEM (with calcium, warm)
4. DMEM with 10% FBS (warm)
5. sterile cell culture dishes (if not tissue culture treated, coat the dishes with 2 % gelatin (just rinse), if not sterile, incubate with ethanol or light-bath with UV lamp for 30 min and then rinse with PBS for 3 times. Not necessary for the current commercial cell culture dish from Fisher).

Procedures

1. Rinse confluent cells with PBS for 3 times
2. Incubate cells with 0.5x trypsin (1 ml for medium dish and 2 ml for large dish), keep in 37^{o}C for 1.5 min, not to over 2 min.
3. Quickly add DMEM (with calcium) to neutralize trypsin (amount does not really matter).
4. Pipet some medium to blow cells into suspension. Double check under microscopy to make sure all the cells are in suspension.
5. Collect cell solution into a tube and centrifuge 1000rpm for 3 min. (keep the balance of centrifuger).

6 During the centrifuging period, take 3 new tissue cultured dishes. Label the dishes with cell name, passage, date, initials of your name.

7 Take out the centrifuged tube containing cells, you should be able to see a whitish pellet at the bottom of the tube. Tilt the tube and aspirate the supernatant with vacuum tip, resuspend the cell pellet with 3 ml 10% FBS DMEM by pipetting up and down 20 times to break cell-cell aggregation. Apply cell solution to labeled dishes, add more 10% FBS DMEM according to the dish size. (2 ml for small dish)

8 Swirl the dish gently to allow the cells to spread evenly throughout the dish.

9 Keep the cell dishes in the incubator supplemented with 5% CO2 at 37^{o}C.

Notes

- The amount of medium can be decided by the size of the cell culture dishes.
- If you are passing cells for transfection, use DMEM without antibiotics.
- If passing cells for transfection, make sure there are not too many cells in the plate. Otherwise, quickly take out some cells before they attach.