Cell Culture Technique For Chondrocytes

Materials:

- 1 DMEM/F12 packet
- Ascorbic-2-phosphate
- Sodium bicarbonate
- Bovine serum albumin (BSA)
- Sodium pyruvate
- Penicillin streptomycin
- Collagenase P
- Fetal Bovine Serum (FBS)
- Trypsin-EDTA
- PBS w/o $CaCl_2$ and $MgCl_2$
- Hemacytometer w/ Coverslip
- Trypan Blue

Procedure

*Always use aseptic technique. Always spray hands with 70% ethanol before going into the incubator as well as the biohood. Spray all bottles with 70% ethanol before putting them in the biohood.

Preparing the Cell Culture Media

1. Put ~800 mL DI water in clean beaker with stir bar.
2. Add following compounds into stirring water:
3. 50 mg ascorbic-2-phosphate
    a. 1.2 g sodium bicarbonate
    b. 1g bovine serum albumin (BSA)
    c. 10 mL sodium pyruvate
    d. 10 mL penicillin streptomycin
    e. Put in more DI water to obtain 1 L of media.
4. pH media to ~7.2
5. Sterile filter into 2 bottles (500 mL media each) – pH will increase after filtering.
6. Label, date and place in refrigerator.
7. Store media at 2-8 degC.

Preparing Collagenase P Solution

1. Weigh all the collagenase P powder and combine into sterile cell culture media and FBS in the following proportions:
    a. 20 mg collagenase P
    b. 10 mL sterile cell culture media
    c. 300 uL FBS
2. Aliquot 10 mL into Falcon tubes.
3. Store collagenase P at -20 degC.

4. *Changing media*

5. Remove flask of cells from incubator and check them under the inverted microscope at 10x magnification.
6. If cells are rounded continue on with changing the media. Put the cells back in the incubator.

7. Place DMEM and FBS in the 37 degC water bath. Remove media after it is warm and remove FBS after it has thawed. Wipe off water and spray with 70% ethanol. Place solutions in biohood.
8. Mix DMEM with 10% FBS in a Falcon tube.
9. Put the flask of cells in the biohood. Place flask upright with the ventilated cap facing upwards.
10. Unscrew the cap of the flask and place aside.
11. Move the media to the corner top of flask – no cell area. Aspirate off the media using a Pasteur pipet attached to a vacuum line. Be careful not to disturb the cells in the flask as well as avoiding the neck of the flask.
12. Pipet necessary volume of DMEM+10% FBS into the flask. (T75 flasks: 10-12 mL , T150: 15-25 mL)
13. Make sure that the cell plated side is covered with media by rocking the flask.
14. Place cap back onto the flask and put it into the incubator.

** Replace media after 2-3 days. The color change of the media from red to orange to yellow (i.e., decreasing in pH) is an indication that the cells are consuming the nutrients. Changing the media before it turns to a yellow color will aid the cells in growing more efficiently (low pH values is detrimental for cell proliferation).

Passaging Cells

** Some steps are similar to the changing media procedure.

1. Remove flask of cells from incubator and check under the inverted microscope at 10x magnification.
2. If cells are 80% confluent (80% of flask contains cells and 20% is open space), passage cells. Put the flask back into the incubator.
3. Place trypsin, collagenase P, DMEM, and FBS in the 37 degC water bath. Remove media after it is warm and remove other solutions after they have been thawed. Place into the biohood.
4. Put flask of cells in the biohood. Place flask upright with the ventilated cap facing upwards.
5. Unscrew the cap of the flask and place aside.
6. Move the media to the corner top of the flask – no cell area. Aspirate off the media.
7. Pipet 1-2 mL of PBS w/o $CaCl_2$ and $MgCl_2$. Rinse cells (face the pipet tip on the cell area side being careful not to disrupt the cells).
8. Rock flask to coat surface. Aspirate off PBS.
9. Pipet 1-2 mL of trypsin into flask. Rock flask to coat surface. Place in incubator for 5-8 minutes.
10. Check to see if cells have been lifted off the surface (either use a microscope or can see a sheet of cells).
11. Make sure that all the cells have been lifted. Gently tap the sides of the flask with palm of hand.
12. If a sheet of cells is present, pipet 1-2 mL of collagenase P to break up the ECM to isolate the cells. Place back into the incubator for 10-20 minutes.
13. Neutralize trypsin and collagenase P with DMEM+10% FBS. The volume of DMEM+10% FBS should be at least 2x the total volume of trypsin+collagenase P. Pipet media into the cell side of the flask to remove all cells from the flask. Pipet the cell solution a couple of times to break up the cell clumps. *Remember the total volume of the cell solution; this will be required to calculate the total number of cells.
14. Pipet the cell solution from the flask into a Falcon tube.
15. Remove 100 uL of the cell solution into a microcentrifuge tube for the cell count.
16. Centrifuge Falcon tube at 300 *g* for 8 minutes. During this time count the cells.
17. Carefully remove Falcon tube from centrifuge, spray and put into the biohood.
18. Put a new flask into the biohood. Unscrew the cap.
19. Aspirate off supernatant with Pasteur pipet.
20. Resuspend in DMEM + 10% FBS.
21. Pipet calculated volume from cell count into new flask to acquire necessary cell density.
22. Put in additional volume of DMEM + 10% FBS into flask. (T75 flasks: 10-12 mL , T150: 15-25 mL)

Counting Cells

1. Obtain 100 uL of cell sample.
2. Dilute sample according by adding in additional media or trypan blue.
3. Pipet 10 uL of sample into hemacytometer. Avoid air bubbles.
4. Count cells in four corners of the grid. **The average count in a square should be ~50. Do not count cells in all four edges of the corner squares (only count for 2). If cell count is too high, dilute further.
5. When using trypan blue, cells with a ring are considered viable while blue dead cells are dead. For cell viability studies, count total number of cells that are alive and dead.

Cell viability calculation: $\frac{\text{total \# of alive cells}}{\text{total \# of cells}} \times 100 = \%\ \text{viability}$

Calculate total number of cells: $\frac{\text{Cell count}}{\text{\# of squares}} \times \text{Diluton} \times 10^4 = \text{\# of cells/mL}$

Total number of cells in Falcon tube = # from (a) x total volume