Week 2: Bovine Stromal Vascular Cells (Fat)

Jan 25th, 2024

General Notes:

* This is the fat cell isolation – other groups will perform isolations for muscle cells for the class

Materials:

* *Tools*:
  + scalpel,
  + tweezers
  + petri dishes
  + flasks
  + 750 um cell strainers
  + 300 um cell strainers
  + 70 um cell strainers
* *Reagents:*
  + DPBS + 10% Anti/Anti (prepared Week 1)
  + Growth Media (prepared Week 1)
  + DMEM + Glutamax
  + 2% Collagenase solution

Protocol Day 1 – Digestion (Thursday 01.25.2024)

1. Place tissue into sterile petri dish with some DPBS + 10% Anti/Anti (~1-3 mL) to keep tissue wet
2. Cut the fat away from any other tissues (e.g., stiff white fascia & connective tissue)
3. Mince with tweezers & scalpel, mincing into 1-3 mm3 chunks, or a thick paste
4. Move tissue to 1x 50 mL falcon tube
5. Add 18 mL DMEM
6. Add 2mL of digestion solution (2% *collagenase II*) for a final concentration of 0.2% collagenase
7. Incubate & shake tube at 37 degrees for 1 hour, inverting the tube to thoroughly mix every 20 minutes.
   1. Can incubate an additional 30 mins if the cells are not adequately suspended.
8. Filter cell suspensions through 750 um cell strainer into a new 50 mL tube
9. Filter cell suspensions through 300 um cell strainers into a new 50 mL tube
10. Filter cell suspensions through 70 um cell strainers into a new 50 mL tube
    1. Take care not to filter through 40 um strainers, as these cells are bigger than BSCs
    2. Change filters as needed as they may get clogged
11. Spin @ 400g for 10 minutes
12. Carefully aspirate the supernatant without disturbing the cell pellet
    1. Note: Aspirate from the top of the liquid down, rather than sticking the aspirating tip all the way into the liquid to avoid leaving residual mature adipose cells, which are buoyant and float at the top of the supernatant.
13. Add 5 mL growth media and pipette up and down to resuspend the cells
14. Count cells
15. Plate ~15,000 cells / cm^2 onto uncoated plates or flasks
16. Add Growth Media to the appropriate volume for the flask
17. Label the flask with your group name, the date, and “SVC P0”
18. Feed regularly using growth media (Sunday, Tuesday, Thursday).

Reference volumes:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Vessel** | **Surface (cm2)** | **~PBS volume** | **~Trypsin volume** | **~Media volume** |
| 6-well plate | 9.6 | 1 mL | 500 uL | 2 mL |
| 12-well plate | 3.5 | 500 uL | 250 uL | 1 mL |
| 24-well plate | 1.9 | 500 uL | 250 uL | 1 mL |
| 48-well plate | 1.1 | 200 uL | 100 uL | 500 uL |
| 96-well plate | 0.32 | 100 uL | 50 uL | 200 uL |
| T-25 | 25 | 3 mL | 1 mL | 5 mL |
| T-75 | 75 | 5 mL | 2 mL | 12 mL |
| T-175 | 175 | 10 mL | 3 mL | 30 mL |