Isolation of Mouse Costal Chondrocytes

Zhaoyang Liu, June 2016

Materials:

Cell Isolation:

70% Ethanol
Filtered PBS
Pronase (Roche, 10165921001) in 1X PBS (2mg/ml)
For one litter: 30mg into 15ml
Collagenase D (Roche, 11088882001) in serum-free DMEM, high glucose (3mg/ml)
For one litter: 90mg into 30ml

Cell Culture:

DMEM, high glucose (Gibco, Cat. No.: 11965092)
Fetal bovine serum (FBS). (SAFC, Cat. No. 12306C)
Penicillin Streptomycin (P/S). (Sigma, P4333)
L-Ascorbic acid (Sigma, A4403.)
100mg power. Soluble: H2O 10 mg/mL (200X). 1ml Aliquot to 1.5ml tube. Store @-20 in dark.
Add 250ul stock to 50ml medium (50ug/ml).

Procedure:

Cell isolation:

1. Sacrifice 2-4 day old neonatal mice by CO2, decapitation, and sterilize torsos in 70% EtOH
2. Isolate anterior rib cages and sternae (removing as much soft tissue as possible). wash 1x with PBS

   The following steps should be done using aseptic technique in cell culture hood.

3. Wash 1x with 1xPBS
4. Put rib cages into a 50ml tube. Digest in 15ml 2mg/ml Pronase solution (30mg pronase in 15ml 1xPBS) at 37C for 1h with constant agitation. (Solution should become cloudy.)
   Pronase solution: dissolve in 1xPBS, then filter sterilize through a 0.2um filter (VWR 28145-501).
   Maximum 8 sternae for 15ml of Pronase solution.
5. Wash 3x with filtered 1X PBS. (In the same 50ml tube.)
6. Digest in 3mg/ml of collagenase D solution laying tube horizontally in 37C cell culture chamber for 1h. Agitate tissue every 30 minutes to ensure adequate digestion.
   Collagenase D solution: dissolve in DMEM, 1%P/S, then filter sterilize through a 0.2um filter.
   Maximum 8 sternae for 15ml of collagenase solution. Make 30ml of collagenase D solution, use 15ml at step 6 and 15ml at step 8.

7. Wash thoroughly 3x with 1X PBS. (In the same 50ml tube.)
8. Transfer sternae to a petri dish (not a cell culture dish!) and add 15ml of Collagenase D solution allowing fibroblasts to adhere, while chondrocytes remain afloat. Incubate at 37C for 4-6h.
9. Transfer cell solution to a new 50ml tube. Pipette solution a few times to disaggregate cells.
10. Filter cell suspension with a 45uM cell strainer into a 50ml tube
11. Centrifuge at 1500 rpm at 4 C for 5min, resuspend with complete growth media (DMEM+10%FBS+1%PS).
12. Seed cells according to desired cell density.
    (Ready to use in 24h: 0.5 million cells/well for 12-well plate or 1 million cells/well for 6-well plate. If seed 0.5 million cells/well for 6-well plate, it may take 2-3 days to reach confluence.)