Osteosarcoma dissociation technique

Date:_____ **Tumor Name**:_____

Diagnosis: Osteosarcoma Primary / Metastasis Treated / Untreated

Clinical History:

Procedure:

Surgeon: Pathologist:

Tumor Processing—Cell Dissociation (OS):

1. Preparation:
    a. Remove Trypsin from -20 freezer and thaw in 37 degree water bath
    b. Remove FBS from -20 freezer and thaw in 37 degree water bath
    c. Prepare 37 degree water bath on magnetic stirrer heating plate
2. Tumor rinsed with $PBS^{-/-}$; use scalpels/tumor press to chop/mince tumor into small pieces/slurry
3. Transfer max 10g of tumor to 100ml screw cap glass bottle
4. Fill with $PBS^{-/-}$ to 100ml mark (1:10 ratio of tissue per volume)
5. Dissociation
    a. Add trypsin to flask with PBS and tumor
        i. Tumor <5g = 600uL; Tumor 5-10g = 900uL; Tumor >10g = 1200uL
    b. Add collagenase type II 200mg (i.e. 2mg/mL) for max 10mg of tumor
    c. Place in warm 37 degree water bath ≤90 min, agitating with magnetic bead at ~200rpm *(aim to have all chunks circulating and lifted off bottom of bottle)*
    d. Remove from water bath once completely dissociated (*goal is dissolution of all white collagen chunks*)
        i. Total time in water bath_____
    e. Once complete stop dissociation by adding to the tube with the tumor
        i. STI (equal amount to trypsin added above)
            1. Tumor <5g = 600uL; Tumor 5-10g = 900uL; Tumor >10g = 1200uL
        ii. DNase – $1/10^{th}$ volume of STI/Trypsin
            1. Tumor < 5g = 60uL; tumor 5-10g = 90uL; tumor > 10g = 120uL
        iii. 1M Magnesium Chloride – $1/10^{th}$ volume of STI/Trypsin
            1. Tumor < 5g = 60uL; tumor 5-10g = 90uL; tumor > 10g = 120uL
        iv. Add DNAse and MagCl in 30uL increments (each) until "slime" (DNA) resolves; mix well and allow to sit at room temp 2-3 min between each addition.
6. Filter with a 70uM cell strainer (with lip) over 50mL Falcon conical tube = _____mL cell suspension
7. Spin cells at 500g (G=RCF) x 5 minutes, aspirate and discard supernatant.
8. Add 10ml of RBC lysis solution to each tube with cells.
9. Incubate at room temp x 10 minutes.
10. Mix solution of PBS/10% FBS: add to each tube to a total of 50ml.
11. Spin at 500g x 5 minutes, aspirate and discard supernatant.

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12. Resuspend cell pellet in PBS/10%FBS.
13. Count cells – if too many large clumps, use 20 gauge syringe and 18 gauge needle. syringe and 18 gauge needle.

Cell Count

Count cells and average 4 boxes - _____ x 10,000 = _____x 10^6 cells/mL

_____x 10^6 cells/mL x _____mL = _____x 10^6 total cells in cell suspension

Cryo suspension

At 6 x 10^6 cells/mL/vial, (total cells) _____x 10^6 cells ÷ 6 = _____cryovials

1. Mix solution of FBS/10%DMSO, total volume = 1mL per cryovial. *(same for cryo pieces)*
2. Spin cells at 1250 RPM (speed) x 10 minutes, aspirate and discard supernatant.
3. Resuspend in FBS/10%DMSO slowly.
4. Aliquot resuspended cells into 1mL per cryovial, place on wet ice.

Preparation of Cells for OS Intra-femoral Injection

1. # of femurs to inject: _____
2. Cell concentration: 1 x 10^6 cells/10uL for OS
3. # of cells needed for injection:

   Number of femurs _____x 1 x 10^6 cells/femur = _____cells
4. Have: _____x 10^6 cells = Need: _____x 10^6 cells

   In: _____ mL **x** mL

   x = _____mL of cell suspension for injections
5. Spin "x mL" cell suspension at 1250 RPM (speed) x 10 min
6. Aspirate and discard supernatant
7. Matrigel needed for injection (OS): _____femurs x 10uL = _____uL
8. Resuspend in matrigel (which was thawed slowly on ice) using a cold pipette tip
9. Place cells in matrigel on ice
10. Set aside cell suspension or matrigel for dead space in syringe:

    Number of needles _____x 60uL cell suspension (dead space) = _____uL
11. Matrigel lot #_____

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Intra-femoral Injection Preparation

Take following materials for procedure: syringe (Hamilton 50uL gastight luer lock), 25G 1/2inch needles (one per mouse), cells suspended in matrigel on ice, _____ml of cell suspension to flush syringe (0.1ml/mouse)

Cryo preserving cells

Resuspend pellet of cells (6 x 10^6 cells/vial for 5 mice) in 10% DMSO/FBS—1ml per vial. Then freeze -80°C in Styrofoam for two days, then transfer into N_2 for long term storage.