**Soft Tissue Sarcoma (STS) dissociation technique**

**Date:**_____ **Tumor Name**:_____

Diagnosis:

History:

Procedure:

Surgeon: Pathologist:

Tumor Inspection and Dissection:

Description:

Weight:

Tumor Processing – Cell Dissociation:

1. Whole tumor (with as little manipulation as possible) placed in tumor press
    a. Press engaged with contents collected in sterile 6cm plate
    b. Use warmed DMEM to rinse residual tumor into tumor press
2. Transfer the pressed tissue to a 50-mL conical vial with medium containing warmed DMEM. If tumor size is >5 grams, split into multiple 50-ml conical tubes as needed for adequate dissociation.
    a. Add 600 microliters of trypsin (if tumor 5-10g add 900uL; Tumor> 10g add 1200uL)
    b. Measure 50 mg of collagenase in eppendorf tube, add 500 microliters of DMEM to eppendorf to dissolve collagenase, and then add to 50-ml conical tube containing tumor/DMEM
    c. Incubate 10min at 37C
    d. Invert gently: tissue "Falls apart"
    e. Check to see if dissociated properly (goal is "slimy" when pipetted near tumor specimen). If too thick add more DMEM and incubate again.
        i. Total time in water bath_____
    f. 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.
3. Filter with 40uM strainer
4. Microscope: determine viability
5. Spin cells at 450g (G=RCF) x 5 minutes, aspirate and discard supernatant
6. Add 10ml of RBC lysis solution to each tube with cells.
7. Incubate at room temp x 10 minutes.

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8. Mix solution of PBS/10% FBS: add to each tube to a total of 50ml.
9. Spin at 450g x 5 minutes, aspirate and discard supernatant.
10. Resuspend cell pellet in PBS/10%FBS.



Preparation of Cells for Injection

1. Count cells and average 4 boxes - _____ x 10,000 = $10^6$ cells/mL
2. _____x $10^6$ cells/mL x _____mL = _____total cells in cell suspension
3. # of mice to inject: _____
4. Matrigel needed for injection: _____mice x 0.1mL = _____mL
5. Account for Dead space in syringe: 0.05ml x 2 (account for possible needle changes) = 0.1mL cell Suspension if not using a no-hub syringe for injection
6. Cell concentration: 1 x $10^6$cells/0.1mL for STS
7. # of cells needed for injection = # of mice _____ x 1 x $10^6$ cells/mouse = _____cells
8. Need 0.1mL cell suspension for dead space
9. Have $\frac{\text{\_\_\_\_\_ x } 10^6 \text{ cells}}{\text{mL}}$ = Need $\frac{\text{\_\_\_\_\_ x } 10^6 \text{ cells}}{\text{x mL}}$

   x = _____mL of cell suspension….Spin "x mL" cell suspension at 1250 RPM x 10 min
10. Aspirate and discard supernatant
11. Resuspend in matrigel (which was thawed slowly on ice) using a cold pipette tip; place on ice
12. Matrigel lot #_____

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

# Childhood Solid Tumor Network
# CSTN



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