Tissue Disaggregation **Kevin Wei MD, PhD**

SAFETY PRECAUTIONS: All work should be performed under the biological safety cabinet observing safety regulations and using sterile technique. Personal protective equipment such as: lab coat, gloves and glasses, should be used during the procedure. Specimens should be handled as if capable of transmitting infection. All contaminated supplies should be properly disposed of in biohazard or sharps containers and liquid waste should be decontaminated with bleach for 20min before being poured down the drain.

NOTE: Pay particular attention to "^HOT SPOT" steps.

MATERIALS AND REAGENTS: HOT SPOT: All reagents and material must be RNase free

Supplies+Equipment	**Reagents**
100X20mm culture dish	1X PBS (ex. Cellgro 21-040-CV or Hyclone)
1.5ml microfuge tubes	0.4% Trypan Blue (Invitrogen 15250-061)
10ml serological pipet	FBS (ex. Gemini 900-108)
Water bath, 37°C	CryoStor CD10 (BioLife Solutions, 210102)
Scalpel	
Surgical forceps, scissors	
70um strainer (ex. Sigma, CD1-1KT)	RPMI 1640 (Invitrogen 11875-093)
50ml conical	DNase I (Roche, 10-104-159-001,
Ice/Bucket or 4°C refrigerator	50mg/ml)*
Centrifuge	Liberase TL (Roche 05401020001,
Mr. Frosty	5 mg/ml)*
Microfuge	Isopropanol
Hemocytometer/microscope	ME (2-Mercaptoethanol)
Waste container for liquid	Qiagen RLT buffer

Aerosol pipet tips (10, 200, 1000) Biosafety carrier
Surgical sample: Specimen jar
Biopsy: Cryovials, 1.5 ml Nalgene # 5000-1020
-80°C freezer
Liquid Nitrogen Storage System
gentleMACS Dissociator (Miltenyi 130-093-235 or 130-095-937)
gentleMACs C Tubes (Miltenyi 130-093-237)

* HOT SPOT: Enzymes should be aliquoted in appropriate volumes for one-time use (ie. no repeated freeze-thaws). After resuspension, Liberase expires within 3 months.

REAGENT PREPARATION:
5% FBS/RPMI:
To 500ml bottle of RPMI 1640 add 25ml of FBS.
Liberase TL:
Dissolve 5 mg of Liberase TL in 1.0 ml RPMI to make 5mg/ml stock. Store at -20°C (100 µl/tube).
DNaseI :

Dissolve 100mg of DNaseI in 2 ml of buffer (20mM Tris-HCl, 1mM MgCl2, 50%glycerol) to make a 50mg/ml stock. Store at -20°C (100 µl/tube)

REAGENT STORAGE: Room Temp: 1X PBS, Trypan blue, Trypan/PBS, RLT, BME
-20°C: aliquot of Liberase TL, DNase I. 4°C: FBS, 5% FBS/RPMI, RPMI 1640, CryoStor.

SPECIMEN STORAGE: Synovial tissue fragments viably frozen in Cryostor at -80°C.

PROCEDURE:
1 Prepare gentleMACS C tubes with 5mL of RPMI (no serum).

2. Synovial tissue samples are stored at -80C

3. Samples are quickly thawed at 37°C and processed individually.

4. Fragments are removed from the cryotube/Cryostor.and placed onto a petri dish. This can be accomplished with narrow forceps.

5. Fragments are picked up with forceps or a needle (do not crush the fragment), briefly touched to absorbent gauze to remove the bulk of Cryostor and placed into gentleMACS C tube with RPMI/Liberase TL.

6. Add 10uL DNAse I (50mg/mL stock) to each C tube for final 100ug/mL.

7. Add 100uL Liberase TL (stock 5mg/mL) to each tube, for final 100ug/mL

8. Macerate the tissues with gentleMACS using the program m_Spleen 04.01.

9. Incubate the tube in a 37°C water bath for 30 minutes.

10. Stop the digestion by adding 10 mL of 5% FBS/RPMI solution.

11. Filter the tissue through the cell strainer (mesh size 70um) into a 50mL conical to remove debris.

12. Centrifuge the filtered cell suspension at 300xg for 5 min.

13. Discard the supernatant and resuspend the pellet in 10 mL 5% FBS/RPMI solution. HOT SPOT: Leave a small volume of supernatant prior to resuspension as pellet can be loose
14. Remove debris by filtering the cell suspension through a cell strainer (mesh size 70um).

15. Centrifuge the cell suspension at 300xg for 5 min.

16. Discard the supernatant and resuspend the pellet in 0.5 mL of 5% FBS/RPMI.

17. Check viability and cell count by diluting in trypan blue. Mix 10uL cells + 10uL Trypan blue. Add 10uL of the mix to hemocytometer.