University of Michigan Comprehensive Cancer Center	SOP-BCR-2.3	**Dissociation of Mammary Tissue**	Author: S. Clouthier Approved: M. Wicha	Rev: 1.0	Issued: 06/15/09 Revised: 7/2/12

1.0 Purpose

The purpose of SOP 2.3 is to provide details on how to dissociate human mammary tissue.

2.0 Scope

SOP 2.3 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	Collagenase	Enzymatic Dissociation	Freezer #2 (026-328S-A)
2.0	Trypsin	Protease	Freezer #2 (026-328S-A)

4.0 Procedures

4.1 Fresh Mammary tissue arrives to NCRC and is placed in Fridge #2 (026-328S-A) until ready for dissociation.

4.2 Transfer tissue to large sterile Petri dish (150mm plate). *If the sample is too large for one plate, use multiple Petri dishes and combine after mincing.*

- Using two scalpels mince tissue into very fine pieces.

4.3 With a sterile funnel pour minced tissue into a dissociation flask, add prepared Collagenase in 1:1 ratio to the tissue (equal amounts of tissue to Collagenase). *Ensure that the tissue is well suspended in the enzyme mixture and the final volume is level with the widest portion of the flask; if necessary use more than one flask.*

4.4 Cover the opening of the flask with sterile aluminum foil and then parafilm.

4.5 Gently dissociate the minced tissue on a rotary shaker until all larger tissue fragments are digested.

- Digestion time will vary. Typically digestion time is 16hours (overnight) for normal human mammary tissue. Longer digestion times may be required for tough fibrous tissue or shorter digestion times for softer tissue.

The Next Day: (Place Trypsin in 37˚C Water Bath to warm)

4.6 After dissociation: Obtain flask(s) from shaker, and spray outside with ethanol. Shake flask gently to mix up the layers, and pour all contents into 500 mL conical tubes and centrifuge for the **1st time at 40 x g for 2 minutes and 20 degrees C.** Then discard the overlying top liquefied fat layer either by pouring slowly and carefully into original flask (pour consistently making certain no cells or the pellet from the bottom of flask are lost), or by putting the tip of a 25 mL pipette down into the brown middle layer and carefully aspirating the brown liquid without disturbing the pellet at the bottom too much, and into 50 mL conical tubes. Centrifuge a **2nd time at the same speed**

4.7 FIBROBLASTS: Label new 50 mL conical tubes “Fibroblasts”.

4.8 Discard the overlying liquefied fat layer again, and put the supernatant (which contains the fibroblasts) into the labeled Fibroblast tubes, careful not to disturb the pellet at the bottom which contains the organoids. Once supernatant is transferred from all tubes into Fibroblast tubes, combine all remaining pellets into 50 mL conical tubes labeled “Organoids”.

4.9 Spin down all fibroblast tubes for a **3rd time at 100 x g for 5 minutes and 4 degrees C**.

4.10 You should see a reddish pellet at the bottom, which are the fibroblasts. Remove and discard the supernatant carefully (the pellet is loose), and rinse the pellet with HBSS (red or white) and combine the pellets into a few 50 mL conical tubes still labeled “Fibroblasts”. After this wash, centrifuge the sample for a **4th time between 1000-1200 rpm for 5 minutes**.

4.11 Discard the supernatant and re-suspend the pellet in HBSS again and then centrifuge **again between 1000-1200 rpm for 5 minutes**. Repeat this wash 2-3 times total, discarding everything but the pellet each time.

4.12 After the third wash, remove the supernatant and re-suspend the pellet in Fibroblast media. Removing the floating chunks in solution using a pipette.

4.13 Plate the fibroblasts in 25 mL of fibroblast media in T150 flasks. After 1.5 hours, change the media!

4.14 EPITHELIAL: Take the pellet from step 4.8 and determine the volume of the pellet, then add three times the amount of **Pre-Warmed Trypsin**. (e.g. 10mL pellet add 30mL Trypsin)

4.15 Gently pipette sample up and down with a 10mL pipette for 1-3 minutes. The sample should become very stringy due to lysis of dead cells and release of DNA. (Remove any more big chunks)

- Place sample in incubator for 5 minutes.

4.16 Add 2% FBS (50mL HBSS, 1mL FBS) to stop trypsin digestion and wash again with HBSS to get rid of FBS. Centrifuge for 5 minutes at 1000-1200 rpm.

5.0 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/2/12	CL	Updated room numbers