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Title: Modeling Breast Cancer in Human Breast Tissue Using a Microphysiological System

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 21
Number of Shots: 51

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Frank Lau:** This is the first protocol that allows human breast tissue to be maintained in culture ex vivo for prolonged periods of time, making it possible to directly study the interactions between human breast tissue and breast cancer.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

1.2. **Frank Lau:** The main advantage of this technique is that it allows macroscopic pieces of human breast tissue – including breast adipocytes, immune cells, vascular structures, ductal structures, and extracellular matrix – to be kept alive ex vivo.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.3. **Frank Lau:** This technique has major implications for several areas of breast cancer research, including personalized medicine, pharmaceutical development, and the pathophysiology of tumor initiation and slower breast cancer processes such as fibrosis and extracellular matrix remodeling.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Introduction of Demonstrator on Camera

1.4. **Frank Lau:** Demonstrating the procedure will be Katherine Hebert and Rakesh Gurrala, a Tulane graduate student and a Tulane medical student from my laboratory.

1.4.1. INTERVIEW: Author saying the above.

1.4.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. All human tissues were collected in accordance with protocol #9189 as approved by the Institutional Review Board Office of LSUHSC.

Protocol

2. Preparation of gelatin plungers and application to the upper cell sheets

2.1. To begin, melt the prepared gelatin solution in a 37-degree Celsius water bath **[1]**. Use a 5 or 10-milliliter serological pipette to dispense 2.5 milliliters of this solution onto each plunger for wells of a 6-well plate **[2]**. *Videographer: This step is important!*

2.1.1. Talent melting the gelatin solution in water bath.

2.1.2. Talent pipetting the gelatin solution onto the plungers.

2.2. Once the gelatin has solidified, move the pNIPAAm-coated *(pronounce "pee-nye-pam-coated")* ASC plates to the biosafety cabinet **[1-TXT]**. Gently place the plungers into the wells of these plates so that the gelatin contacts the ASCs **[2]**. *Videographer: This step is important!*

2.2.1. Talent placing the ASC plates in the biosafety cabinet. **TEXT: pNIPAAm- poly N-isopropylacrylamide; ASC- adipose-derived stem cell**

2.2.2. Talent placing the plungers in the wells of the ASC plates.

2.3. Place a metal washer on the plunger to weigh it down so that the gelatin is in direct contact with the ASC sheet for 30 minutes at room temperature **[1]**.

2.3.1. Talent placing metal washer on the plunger.

2.4. Gently move the plate with the plungers into the sterile box in the biosafety cabinet **[1]** and place the lid on it **[2]**. Move the box to a 4-degree fridge or place the plate on ice in an ice bucket in the biosafety cabinet for 30 minutes **[3]**.

2.4.1. Talent moving the plate in sterile box.

2.4.2. Talent placing lid on the box.

2.4.3. Talent moving the sterile box into the 4 degree fridge.

3. Processing of human breast tissue

3.1. In a biosafety cabinet, wash the human breast tissue 3 times with 10 milliliters of sterile PBS **[1-TXT]**. Use sterile forceps and a razor blade to coarsely mince the BC-MPS and try to remove as much fascia and connective tissue as possible **[2-TXT]**. *Videographer: This step is important!*

3.1.1. Talent washing the HBT with PBS.

3.1.2. Talent mincing the BC-MPS using forceps and blade. **TEXT: BC-MPS-Microphysiological systems for breast cancers**

3.2. Once the connective tissue has been removed, use a sterile razor blade to finely mince the tissue until it has a homogenous liquid consistency **[1]**. Cut the tip of a p1000 pipette tip to assist in pipetting the minced tissue **[2]**. *Videographer: This step is difficult*

3.2.1. Talent mincing the connective tissue with blade.

3.2.2. Talent cutting the tip of the p1000 pipette tip.

3.3. In a 1.5-milliliter tube, combine the minced tissue, cancer cell lines, and BC-MPS as described in the text manuscript **[1]**. Move the ASC plate that will be used for the bottom cell sheet from the incubator to the biosafety cabinet **[2]** and aspirate the media from the plate **[3]**. *Videographer: This step is important!*

3.3.1. Talent mixing the components in the tube.

3.3.2. Talent moving out the ASC plate from the incubator to the biosafety cabinet.

3.3.3. Talent aspirating media from the bottom ASC plate.

3.4. Pipette the prepared breast tissue mixture onto the center of the well of the bottom ASC plate using the cut pipette tip **[1]**. Move the box containing the upper ASC plate with plungers to the biosafety cabinet **[2]**. Gently remove the gelatin plungers from the pNIPAAm (*'pee-nye-pam'*) -coated plate **[3]** and place them on top of the tissue mixture **[4]**. *Videographer: This step is difficult and important!*

3.4.1. Talent pipetting the mixture onto the wells of bottom ASC plate.

3.4.2. Talent moving the ASC plate with plunger to the biosafety cabinet.

3.4.3. Talent gently removing the gelatin plungers.

3.4.4. Talent placing the plungers on top of the HBT mixture.

3.5. Add BC-MPS media to the well **[1]** and carefully move the bottom ASC plates with the BC-MPS mixture and plungers to the sterile plastic box **[2]**. Place the lid of the box on for transport, taking care to avoid contamination of the culture **[3]**.

3.5.1. Talent adding BC-MPS media to the well.

3.5.2. Talent moving the ASC plate with HBT mixture and plungers to the sterile plastic box.

3.5.3. Talent replacing the lid of the plastic box.

3.6. Incubate the bottom plate in the box in a 37-degree Celsius incubator until the gelatin is melted and the top ASC layer has begun to adhere to the bottom layer **[1]**, then move the box with the plates to the biosafety cabinet **[2]**.

3.6.1. Talent incubating the box containing bottom plate in 37-degrees.

3.6.2. Talent moving the box with plates to biosafety cabinet.

3.7. Gently remove the plungers from the bottom plates to observe the tissue with the cancer cells anchored to the bottom of the well **[1]**. *Videographer: This step is important!*

3.7.1. Talent removing the plungers from the bottom plates.

3.8. Place the lid of the 6-well plate back onto the bottom plate **[1]** and incubate at 37-degrees to completely melt the gelatin and allow the top layer to anchor to the bottom layer **[2]**. Gently move the plates to a biosafety cabinet **[3]** and aspirate the media from the edge of the well with a 10-milliliter serological pipette **[4]**.

3.8.1. Talent placing the lid onto the bottom plate.

3.8.2. Added shot: Talent moving plate to 37 -degrees. The plate sits in the incubator until gelatin is melted. Then 3.8.3 is performed. NOTE: May be slated differently

3.8.3. Talent moving the plate to biosafety cabinet.

3.8.4. Talent aspirating the media.

3.9. Add 2 milliliters of fresh media onto the edge of each well to avoid dislodging the tissue **[1]**. Maintain the BC-MPS at 37 degrees Celsius and 5% carbon dioxide for the desired length of time, changing the media every 2 to 3 days **[2]**.

3.9.1. Talent adding fresh media to each well.

3.9.2. Talent moving the BC-MPS to the 37-degrees.

4. Digestion of BC-MPS for analysis

4.1. Move the plate to the biosafety cabinet when the BC-MPS is ready to be analyzed **[1]**. Remove media with a serological pipette to avoid accidental dislodging of any tissue **[2]**. Add 1 volume of PBS to each well **[3]**, then remove the PBS with a serological pipette **[4]**.

4.1.1. Talent moving the plate to biosafety cabinet from incubator.

4.1.2. Talent removing media from the plate.

4.1.3. Talent adding PBS to each well.

4.1.4. Talent removing PBS.

4.2. Add 1 milliliter of cell disassociation solution to each well **[1]** and move the plate back to the incubator for 5 minutes to allow the cells to detach **[2]**. After incubation, use a cell scraper **[3]** to completely detach the cells and the tissue from the culture plate in a biosafety cabinet **[4]**.

4.2.1. Talent adding cell dissociation solution in each well.

4.2.2. Talent placing the plate in the incubator.

4.2.3. Added shot: Talent moving the plate to the biosafety cabinet. NOTE: May be slated differently

4.2.4. Talent scrapping the cells and tissue from the culture plate.

4.3. Transfer the solution with the tissue to a 15-milliliter conical tube **[1]** and collect any remaining cells **[2]** by adding 2 milliliters of PBS **[3]**. Wrap the tube with aluminum foil if the cells are fluorescent **[4]**.

4.3.1. Talent transferring the tissue solution in conical tube.

4.3.2. Added shot: Talent adding PBS to each well. NOTE: May be slated differently

4.3.3. Talent aspirating remaining cells from the well using PBS.

4.3.4. Talent wrapping the tube with aluminum foil.

4.4. Incubate the tube at 37 degrees under constant agitation in an orbital shaker at 1 times *g* for 10 to 20 minutes to completely dissociate the cells from the tissue **[1]**.

4.4.1. Talent incubating the tube in orbital shaker.

4.5. In a biosafety cabinet, use a serological pipette to disrupt any remaining clumps of cells in the tube **[1]** and filter the sample through a 250-micrometer tissue strainer

into a new 15-milliliter tube **[2-TXT]**. Rinse the strainer with 1 milliliter of PBS to collect any remaining cells **[3]**.

4.5.1. Talent disrupting clumps of cells using pipette.

4.5.2. Talent filtering the sample through a filter. **TEXT: Pipette slowly to avoid overflow**

4.5.3. Talent rinsing the strainer with PBS.

4.6. Centrifuge the samples at 500 times *g* at room temperature for 5 minutes to separate the adipocytes, which will be floating in the top layer, from the cancer cells and ASCs mixed together in a pellet **[1]**. Transfer the adipocyte layer to a new tube using a blunt cut pipette tip **[2]**.

4.6.1. Talent centrifuging the tube.

4.6.2. Talent transferring the adipocytes top layer into a new tube.

4.7. Centrifuge the sample again **[1]** and use a syringe and a needle to remove the remaining solution from below the adipocytes **[2]**.

4.7.1. Talent centrifuging the sample.

4.7.2. Talent removing the remaining solution using syringe and a needle.

4.8. Aspirate the remaining solution from the tube containing the ASCs and cancer cells without disrupting the cell pellet **[1-TXT]**. Resuspend the pellet in PBS or BC-MPS media **[2]** and use flow cytometry to sort the cells based on fluorescence **[3]**.

4.8.1. Talent aspirating the remaining solution. **TEXT: Separate ASCs from fluorescent protein transfected cancer cells**

4.8.2. Talent adding PBS or BC-MPS.

4.8.3. Talent takes plate and leaves lab

Results

5. Analysis of BC-MPS culture

5.1. The striated pattern of the confluent ASC sheet is shown here **[1]**. The buoyant human breast tissue was still stably anchored by the ASC cell sheets to the bottom of the well after 14 days of culture **[2]**.

5.1.1. LAB MEDIA: Figure 2A.

5.1.2. LAB MEDIA: Figure 2B.

5.2. Fluorescent microscopy images demonstrate the stability of the BC-MPS with MDA-MB-231 expressing RFP cell lines after 14 days **[1]** and with triple negative tumor explant-PDX for at least 6 days **[2]**.

5.2.1. LAB MEDIA: Figure 2C.

5.2.2. LAB MEDIA: Figure 2D.

5.3. BC-MPS containing breast tissue was cultured in vitro for 14 days **[1]**, stained, and imaged to demonstrate the native elements of the tissue at 100x **[2]** and 20x magnification **[3]**.

5.3.1. LAB MEDIA: Figure 3A.

5.3.2. LAB MEDIA: Figure 3B. *Video editor: Use arrows to label the purple stained region as native collagen and blue as reticular fibers.*

5.3.3. LAB MEDIA: Figure 3C.

5.4. Staining of the macrophage markers was used to show the preservation of primary macrophages after 3 and 7 days in culture **[1]**.

5.4.1. LAB MEDIA: Figure 3D.

5.5. Flow cytometry analysis of Bodipy-stained MDA-MB-231 cells after 14 days indicate minimal lipid accumulation in 2D culture **[1]** and extensive lipid accumulation in BC-MPS **[2]**. The proportion of lipid-positive MDA-MB-231 cells was 26.2-fold greater in BC-MPS than 2D culture **[3]**.

5.5.1. LAB MEDIA: Figure 4A. *Video editor: first focus on the cells in B2, then gradually focus on the cells B1.*

5.5.2. LAB MEDIA: Figure 4B. *Video editor: first focus on the cells in B2 and gradually focus on the cells in B1.*

5.5.3. LAB MEDIA: Figure 4C.

5.6. Cells cultured in BC-MPS displayed increased lipid droplets compared to cells cultured in standard 2D culture **[1]**.

5.6.1. LAB MEDIA: Figure 4 D and E. *Video editor: Label E "BC-MPS" and D "2D culture".*

5.7. Time-lapse images of RFP-labeled BC-MPS with MDA-MB-231 cells showed amoeboid movement of 231 cells with pseudopods and high motility **[1]**.

5.7.1. LAB MEDIA: Figure 5. *Video editor: label yellow asterisk as "mitotic event", yellow box as "reference cell debris" and blue arrow as "cell movement".*

Video editor: also play the video file Jove Brown Movie 1.avi in the inset.

Conclusion

6. Conclusion Interview Statements

6.1. **Frank Lau:** The breast tissue must be minced small enough and separated enough to be distributed across the bottom of the well. Breast tissue is very buoyant, and if the pieces are too large or clustered too closely together, the ASC cell sheets won't be able to anchor the breast tissue to the bottom of the well.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1 and 3.2*

6.2. **Frank Lau:** The resulting breast tissue-cancer constructs can undergo enzymatic digestion to isolate particular cell types of interest. This would allow key questions to be answered, such as how cancer cells in breast tissue respond differently to chemotherapy compared to traditional 2D culture, or organoids.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.