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Title: Modeling the Effects of Hemodynamic Stress on Circulating Tumor Cells Using a Syringe and Needle

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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: 12
Number of Shots: 29

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Michael Henry:** This protocol exposes cancer cells in a suspension to brief pulses of fluid shear stress to mimic certain aspects of how metastatic cancer cells are exposed to hemodynamic forces while in the circulatory system.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.3.1 for 'fluid shear stress'*

1.2. **Michael Henry:** It is a relatively simple technique to apply brief pulses of high-level fluid shear stress.

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

OPTIONAL:

1.3. **Devon Moose:** When attempting this protocol, be careful around uncapped needles and do not bend them as it will change the applied fluid shear stress. This procedure may produce aerosols, so use appropriate safety measures.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1 for 'around uncapped needles'*

Protocol

2. Cell Preparation

2.1. To begin, release 70 to 90% confluent PC-3 cells from the tissue culture dish **[1]** by aspirating the growth medium **[2]** and washing the 10-centimeter dish with 5 milliliters of calcium and magnesium-free PBS **[3]**. Then, aspirate the PBS **[4]** and add 1 milliliter of 0.25% trypsin **[5]**.

2.1.1. WIDE: Talent working with the tissue culture dish.

2.1.2. Talent aspirating growth medium.

2.1.3. Talent washing cell dish.

2.1.4. Talent aspirating PBS.

2.1.5. Talent adding trypsin.

2.2. Post trypsinization, observe the detachment of the cells under an inverted microscope **[1]**. To inhibit the trypsin, add 5 milliliters of DMEM:F12 *(pronounce D-M-E-M-F-12)* medium containing 10% FBS **[2]**.

2.2.1. Talent working with the microscope.

2.2.2. Talent adding DMEM:F12 medium to cells.

2.3. Next, place the cell suspension into a conical tube and determine the cell concentration and total cell number **[1]**.

2.3.1. Talent placing cells in a tube and determining cell number.

2.4. Centrifuge the cell suspension at 300 × *g* for 3 minutes **[1]**, then aspirate the supernatant and resuspend the pellet in a serum-free tissue culture medium **[2-TXT]**.

2.4.1. Talent placing cells in a centrifuge and closing the lid.

2.4.2. Talent resuspending the pellet. **TEXT: 5 × 10^5 cells/mL**

3. Fluid Shear Stress (FSS) Exposure

3.1. Cut a round-bottom of 14-milliliter polystyrene tube at the 7-milliliter line **[1]** and place the mixed cell suspension into the cut tube **[2]**. Separately collect static control samples of cells before FSS exposure to use for performing assays **[3]**.

3.1.1. Talent cutting tube.

3.1.2. Talent placing cell suspension in a tube.

3.1.3. Shot of static control samples in labeled tubes/containers.

3.2. To expose the remaining cell suspension sample to FSS, draw the cell suspension into a 5-milliliter syringe and attach a 30-gauge half-inch needle **[1]**. Place and secure the syringe with an uncapped needle onto a syringe pump **[2]** and set the flow rate to achieve the desired level of FSS **[3]**.

3.2.1. Talent drawing cell suspension into a syringe and attaching a needle. *Videographer: This step is important!*

3.2.2. Talent placing syringe onto the pump. *Videographer: This step is important!*

3.2.3. Talent setting the flow rate of the pump. *Videographer: This step is important!*

3.3. Run the syringe pump and collect the sheared sample in the cut tube at an approximately 45-degree angle to reduce foaming **[1-TXT]**. Carefully remove the syringe from the syringe pump **[2]** and use pliers to remove the needle, taking care not to touch it **[3]**.

3.3.1. Shot of syringe pump in work and talent collecting sample in the cut tube. **TEXT: Collection depends on viability assay/downstream assay** *Videographer: This step is important!*

3.3.2. Talent removing the syringe from the pump. *Videographer: This step is important!*

3.3.3. Talent removing the needle. *Videographer: This step is important!*

3.4. Repeat the procedure until the cell suspension has been exposed to the desired number of pulses of FSS **[1]**.

3.4.1. Talent refilling the syringe and attaching the needle using pliers.

4. Viability Measurement

4.1. Perform viability assays with the static samples before exposing the cells to FSS **[1]**. For enzymatic assays, transfer 100-microliter aliquots from the static samples in duplicates into a 96-well plate **[2]**.

4.1.1. WIDE: Talent working with static samples collected in labeled containers. Use 3.1.3 for 4.1.1 – 4.2.1

4.1.2. Talent removing the aliquots of the static sample.

4.2. Then, collect 100 microliters of samples after FSS exposure and place them in a 96-well plate **[1-TXT]**. Add 20 microliters of a 0.15-milligram per milliliter resazurin solution to each sample well and to wells containing 100 microliters of medium alone **[2]**.

4.2.1. Talent collecting samples after FSS exposure and placing them into well. **TEXT: 1, 2, 4, 6, 8, and 10 pulses of FSS exposure**

4.2.2. Talent adding resazurin solution to wells.

4.3. Incubate the well plates in a 37-degree Celsius tissue culture incubator for 2 hours **[1]**. Then, measure the fluorescence and absorbance using a plate reader **[2-TXT]** and obtain the percentage of viable cells by comparing the averaged signal from each of the FSS-exposed samples to the averaged static control sample **[3]**.

4.3.1. Talent placing plates in an incubator.

4.3.2. Talent working with the plate reader/ measuring fluorescence. **TEXT: 579 excitation/ 584 emission**

4.3.3. Talent working with the results on a spreadsheet.

4.4. Similarly, collect aliquots from static samples to perform flow cytometry and clonogenic assays as described in the text manuscript **[1]**.

4.4.1. Talent collecting aliquots. Use 3.4.1

Results

5. Results: Fluid Shear Stress Resistance of Mammary Epithelial Cancer Cells

5.1. In the representative analysis, the viability of syngeneic BALB/c *(pronounce Balb-C)* mammary epithelial cancer cells was assessed using resazurin conversion after exposing cells to a number of FSS pulses **[1-TXT]**.

5.1.1. LAB MEDIA: Figure 1. **TEXT: 30 G needle, 10 pulses at 250 mL/s.** *Video Editor: Emphasize titles of the x and y-axis.*

5.2. Even though each cell line displayed different resistance profiles, there was no significant difference in viability after 10 pulses of FSS exposure **[1]**.

5.2.1. LAB MEDIA: Figure 1. *Video Editor: Emphasize titles for data lines (right corner) and three data lines.*

5.3. Additional cancer cell lines from a variety of tissue origins demonstrated the viability of more than 20% after 10 pulses of FSS **[1-TXT]**, except for MiaPaCa2 *(pronounce M-I-A-P-A-C-A-2)* cells, which showed a viability of less than 10% due to sensitivity towards mechanical destruction from FSS **[2]**.

5.3.1. LAB MEDIA: Table 2. **TEXT: 30 G needle, 10 pulses at 250 mL/s**

5.3.2. LAB MEDIA: Table 2. *Video Editor: Emphasize row 17.*

Conclusion

6. Conclusion Interview Statements

6.1. **Devon Moose:** When collecting the static sample prior to applying fluid shear stress, ensure that the cell suspension is homogenously mixed. Carefully remove the needle with pliers and do not bend or touch the needle.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.3 for 'static sample', 3.3.3 for 'remove the needle'*

6.2. **Devon Moose:** In addition to measuring cell viability, one can evaluate changes in gene expression, cell signaling, proliferation, and migration. Using this as a model of exposure to fluid shear stress one can study both how cancer cells resist destruction by fluid shear stress and evaluate the effects of fluid shear stress on the biology of cancer cells.

6.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.2 for 'of exposure to fluid shear stress'*

6.3. **Michael Henry:** This technique has been used by our laboratory and others to explore the effects of fluid shear stress on circulating tumor cells. These studies have shown that fluid shear stress rapidly alters the mechanical properties of cancer cells and that this contributes to the ability of cancer cells to resist destruction by fluid shear stress.

6.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1 for 'properties of cancer cells'*