Submission ID #: 61606
Scriptwriter Name: Bridget Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18789878

Title: A Biomimetic Model for Liver Cancer to Study Tumor-Stroma Interactions in a 3D Environment with Tunable Bio-Physical Properties

Authors and Affiliations: C. Calitz[1], N. Pavlović [1], J. Rosenquist[2], C. Zagami[1], A. Samanta[2], and F. Heindryckx[1]

[1]Department of Medical Cell Biology, Uppsala University
[2]Polymer Chemistry, Department of Chemistry-Ångström Laboratory, Uppsala University

Corresponding Author:
Femke Heindryckx
femke.heindryckx@mcb.uu.se

Co-Authors:
carlemi.calitz@mcb.uu.se
natasa.pavlovic@mcb.uu.se
jenny.rosenquist@kemi.uu.se
claudia.zagami.3161@student.uu.se
ayan.samanta@kemi.uu.se

Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **Y, 3 km**

Protocol Length
Number of Shots: **48**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Femke Heindryckx**: This model recapitulates both the premalignant and tumor microenvironments by incorporating physiologically relevant hydrogels with tunable stiffness and hepatocellular and stroma associated cell lines **[1]**.

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

REQUIRED:

1.2. **Carlemi Calitz**: This model is modular and cost-effective, can be prepared with basic equipment and readily available materials, and can be used to study complex tumour stroma interactions **[1]**.

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

OPTIONAL:

1.3. **Femke Heindryckx**: This tumor stroma interaction model can provide insight into hepatocarcinogenesis, which is of considerable importance from both a mechanistic and treatment perspective **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*

Introduction of Demonstrator on Camera

1.4. **Femke Heindryckx**: Demonstrating the procedure with Carlemi Calitz will be Jenny Rosenquist, a doctoral student from the Ångström laboratory **[1][2]**.

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

Protocol

2. Fibrinogen Stock Solution

2.1. To prepare 10 milliliters of an 80-milligram/milliliter fibrinogen stock solution, first add 2.21 grams of calcium chloride **[1]** and 5 milligrams of aprotinin to individual, 20-millliter volumes of distilled water **[2]**.

2.1.1. WIDE: Talent adding CaCl2 to water, with CaCl2 containers visible in frame

2.1.2. Talent adding aprotinin being added to water, with aprotinin container visible in frame

2.2. Then add 2.051 milliliters of the aprotinin stock solution **[1]** and 100 microliters of the calcium chloride stock solution to 7.849 milliliters of PBS **[2]**.

2.2.1. Talent adding aprotinin solution to tube, with aprotinin and PBS containers visible in frame

2.2.2. Talent adding calcium chloride to tube, with CaCl2 and PBS containers visible in frame

2.3. Next, incrementally add 800 milligrams of fibrinogen **[1]** and 200 milligrams of sodium chloride to the solution without stirring or shaking **[2]** and place the tube of fibrinogen stock solution horizontally on a shaker **[3]**.

2.3.1. Talent mixing fibrinogen and sodium *Videographer: Important step*

2.3.2. Talent adding increments of sodium chloride to tube, with sodium chloride container visible in frame. Author NOTE: The mixture is added to the tube here *Videographer: Important step*

2.3.3. Talent placing tube horizontally onto shaker

2.4. After 2-5 hours of shaking at 300 revolutions per minute, filter the resulting solution to remove any clumps that formed during the preparation **[1]**.

2.4.1. Talent filtering solution

3. Insert Coating

3.1. For cell culture insert collagen coating, in a biosafety cabinet, use sterilized tweezers to invert the inserts onto the lid of the cell culture plate **[1]**.

3.1.1. WIDE: Talent inverting insert(s) onto plate lid

3.2. Next, mix 115 microliters of glacial acetic acid with 25 milliliters of distilled water **[1]** before adjusting the solution with additional distilled water to a final volume of 100 milliliters before filtering **[2]**.

3.2.1. Talent adding acid to water, with acid container visible in frame

3.2.2. Talent adding water to 100 milliliters, with filter visible in frame

3.3. To prepare 2 milliliters of a 100 microgram/milliliter collagen solution, add 40 microliters of a 5 milligram/milliliter collagen solution to 1.96 milliliters of the 20-millimolar glacial acetic acid stock solution **[1]** and add 100 microliters of the resulting collagen solution to the bottom of each insert **[2]**.

3.3.1. Talent adding collagen to acid solution, with collagen and acid containers visible in frame

3.3.2. Collagen being added to insert(s) *Videographer: Important step*

3.4. After 2-3 hours of air drying, wash the inserts by briefly placing them collagen coating-side down in individual wells of a 12-well plate containing 1 milliliter of fresh PBS per well per wash **[1]**.

3.4.1. Talent placing insert(s) into well(s), with PBS container visible in frame NOTE: Put 3.6, VO and shot, before 3.4.1

3.5. After the last wash, place the inserts back onto the plate lid for another 1-2 hours of air drying **[1]**.

3.5.1. Talent placing inverted insert(s) onto lid

3.6. When the inserts have dried, place a custom 3D printed spacer over the inserts **[1]**.

3.6.1. Talent placing spacer over inserts NOTE: This should go before 3.4.1.

3.7. Then cover the inverted inserts with the bottom of the plate **[1]** and place the inserts into the cell culture incubator **[2]**.

3.7.1. Talent placing plate over inserts

3.7.2. Talent placing inserts into incubator

4. Liver Cell Seeding

4.1. Before seeding the cells, add 3.99 grams of sodium hydroxide to 100 milliliters of distilled water **[1]** and filter the solution through a 0.22-micrometer syringe filter **[2]**.

4.1.1. WIDE: Talent adding NaOH to water, with NaOH container visible in frame

4.1.2. Talent filtering solution

4.2. Next, wash T175 (T-one-seventy-five) hepatic stellate and liver carcinoma cell cultures two times with 10 milliliters of PBS per wash **[1]** before treating the cells with 6 milliliters of trypsin for 4 minutes at 37 degrees Celsius **[2]**.

4.2.1. Talent washing flask(s), with PBS container visible in frame

4.2.2. Talent adding trypsin to flask, with trypsin container visible in frame

4.3. When the cells have detached, inactivate the enzymes with 6 milliliters of 37-degree Celsius 10% DMEM (D-M-E-M) **[1]** and transfer each cell culture to individual 15 milliliter tubes **[2]**.

4.3.1. Talent adding medium to flask(s), with medium container visible in frame

4.3.2. Talent adding cells to tube(s)

4.4. Sediment the cells by centrifugation **[1-TXT]** and resuspend the pellets in 5 milliliters of pre-warmed 10% DMEM per tube **[2]**.

4.4.1. Talent placing tube(s) into centrifuge **TEXT: 3 min, 300 x g, RT**

4.4.2. Shot of pellet if visible, then medium being added to tube, with medium container visible in frame

4.5. After counting, dilute both cell populations to 1 x 10^6 cells per milliliter of medium concentrations **[1]** and collect the cells by centrifugation **[2]**.

4.5.1. Talent adding medium to tube(s), with medium container visible in frame

4.5.2. Talent adding tube(s) to centrifuge

4.6. After removing the supernatants, add the appropriate volume of pre-warmed 10% DMEM to the cells as indicated in the Table **[1]** and neutralize the appropriate volume of 4-degree Celsius collagen with 10 microliters/milliliter of 4-degree Celsius sodium hydroxide **[2]**.

4.6.1. LAB MEDIA: Table 2 *Video Editor: please emphasize 10% DMEM column*

4.6.2. Talent neutralizing collagen

4.7. Add the chilled neutralized collagen to the cell suspension. The medium will turn yellow **[1]**. After mixing the suspension thoroughly with a cut pipette tip, the medium will turn bright pink **[2]**.

4.7.1. Talent adding collagen to cells/medium turning yellow NOTE: 4.7.1 and 4.7.2 shot together *Videographer: Important/difficult step*

4.7.2. Medium being mixed/turning pink *Videographer: Important/difficult step*

4.8. Add the appropriate volume of 37-degree Celsius fibrinogen as indicated in the Table **[1]** and use a cut pipette tip to thoroughly mix the suspension **[2]**.

4.8.1. LAB MEDIA: Table 1 *Video Editor: please emphasize fibrinogen column*

4.8.2. Talent mixing tube contents *Videographer: Important/difficult step*

4.9. After mixing, add 0.1 kilo international units of thrombin for each 10 milligrams of fibrinogen to each tube **[1]** and use a modified 200-microliter pipette tip to add 200 microliters of the cell suspension to the bottom of each insert **[2]**.

4.9.1. Talent adding thrombin to suspension, with thrombin container visible in frame NOTE: 4.9.1 and 4.9.2 shot together *Videographer: Important/difficult step*

4.9.2. Talent adding suspension to insert(s) *Videographer: Important step*

4.10. Allow the gels to crosslink for 15 minutes at room temperature **[1]** before gently placing the bottom section of the plate over the gels **[2]**. Then place the inserts into the incubator for additional crosslinking at 37 degrees Celsius for 45 minutes **[3]**.

4.10.1. Talent setting timer, with inserts visible in frame

4.10.2. Talent placing plate over inserts *Videographer: Important step*

4.10.3. Talent placing inserts into incubator

4.11. At the end of the incubation, place the inserts into individual wells of a 12-well plate **[1]** and add 2 milliliters of pre-warmed 10% DMEM to each well **[2]**.

4.11.1. Talent placing insert(s) into well(s)

4.11.2. Talent adding medium to well(s)

5. Endothelial Cell Seeding

5.1. For the seeding of endothelial cells onto the inserts, wash a T175 human vascular endothelial cell culture with 10 milliliters of PBS **[1]** before detaching the cells with 37-degree Celsius trypsin as demonstrated **[2]**.

5.1.1. WIDE: Talent adding PBS to flask, with PBS container visible in frame

5.1.2. Talent adding trypsin to flask, with trypsin container visible in frame

5.2. Resuspend the cells in 5 milliliters of endothelial growth medium for counting **[1]** and resuspend the cells to a 1 x 10^4 cells/milliliter of endothelial growth medium concentration **[2]**.

5.2.1. Talent adding medium to cells, with medium container and hemocytometer visible in frame

5.2.2. Talent adding medium to cells, with medium container visible in frame

5.3. Then add 500 microliters of cells to the top each insert **[1]** and place the plate in the cell culture incubator for 21 days **[2-TXT]**.

5.3.1. Talent adding cells to insert(s)

5.3.2. Talent placing plate into incubator **TEXT: Refresh medium every other day**

5.4. To measure the storage moduli of the gel formulations, use a rheometer sixty minutes after seeding to perform frequency sweeps from 0.1-20 hertz at 0.267% and 37 degrees Celsius with a constant axial force of 0.1 Newtons using an 8-millimeter diameter parallel plate with stainless steel geometry **[1]**.

5.4.1. Talent measuring stiffness values

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?

2.3., 3.3.2., 4.7.-4.10.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
4.7-4.10. you have to really work fast and ensure that you are well prepare in advance, please see the following https://youtu.be/_yYu2jSwhu0

Results

6. Results: Representative Biomimetic Liver Cancer Model Preparations and Analyses

6.1. In this representative analysis, ten formulations of the fibrinogen **[1]** and collagen hydrogel combinations were prepared **[2]** to determine which formulations could mimic liver stiffness similar to that observed during the development of hepatocellular carcinoma **[3]**.

6.1.1. LAB MEDIA: Table 2
6.1.2. LAB MEDIA: Table 2 *Video Editor: please emphasize Fibrinogen column*
6.1.3. LAB MEDIA: Table 2 *Video Editor: please emphasize Collagen column*

6.2. The storage modulus of each concentration was then determined using a rheometer **[1]**.

6.2.1. LAB MEDIA: Figure 8 *Video Editor: please emphasize add data bars or no animation*

6.3. From these ten formulas **[1]**, the 2 milligrams/milliliter of collagen type one to 10 milligrams/milliliter of fibrinogen, which corresponds to the liver stiffness values at the onset of fibrosis **[2]**, 2 milligrams/milliliter of collagen type one to 30 milligrams/milliliter of fibrinogen, which corresponds to cirrhosis **[3]**, and 2 milligrams/milliliter of collagen type one and 40 milligrams/milliliter of fibrinogen, which corresponds to hepatocellular carcinoma, were selected **[4]**.

6.3.1. LAB MEDIA: Figure 9
6.3.2. LAB MEDIA: Figure 9 *Video Editor: please emphasize blue data bar*
6.3.3. LAB MEDIA: Figure 9 *Video Editor: please emphasize green data bar*
6.3.4. LAB MEDIA: Figure 9 *Video Editor: please emphasize red data bar*

6.4. AlamarBlue analysis **[1]** showed an overall reduced cell viability within 2D co-cultures that is lower than expected based on the known reported inhibitory concentration values **[2]** compared to an untreated control **[3]**.

6.4.1. LAB MEDIA: Figure 10
6.4.2. LAB MEDIA: Figure 10 *Video Editor: please emphasize white IC75, 50, and 25 data bars and/or add asterisks over these bars*
6.4.3. LAB MEDIA: Figure 10 *Video Editor: please CTRL data bar*

6.5. In the 3D model, however, an increase in doxorubicin resistance was observed **[1]**.

6.5.1. LAB MEDIA: Figure 10 *Video Editor: please emphasize red IC75, 50, and 25 data bars and/or add asterisks over these bars*

Conclusion

7. Conclusion Interview Statements

7.1. **Carlemi Calitz**: Be sure to work quickly when seeding the hydrogel cell suspension onto the inserts, as a streamlined workflow is essential to the success of the protocol **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (4.7.-4.10.)

7.2. **Femke Heindryckx**: Optimization of the clearing and staining techniques will allow the visualization of tumor stroma interactions at the transcriptional and translational levels **[1]**. NOTE: Text changed

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera *Videographer: Can cut for time*