FINAL SCRIPT: APPROVED FOR FILMING

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Title: Microbioreactor-Based Production of Anchorage-Dependent Mesenchymal Stromal Cells Primed for Acute Respiratory Distress Syndrome

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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? **YES, all done**

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

Current Protocol Length
Number of Steps: 23
Number of Shots: 51

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Brandon Krupczak:** We study how cells interact with their environment, including when producing or manufacturing cells as therapies. Here, we addressed questions about how anchorage-dependent cells like MSCs – mesenchymal stromal or stem cells – can be produced to overcome some barriers to clinical translation.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:5.2*

What are the most recent developments in your field of research?

1.2. **Brandon Krupczak:** Growing recognition that cell population variability affects potency of the final cell product, for every batch or person. These challenges can even affect regulatory approval of some cell therapies.

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

What research gap are you addressing with your protocol?

1.3. **Julia Dias:** We target a gap that can achieve a high number of cells per milliliter of fluid, at high control of the environment - and in a way that can be scaled up.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.7*

What advantage does your protocol offer compared to other techniques?

1.4. **Julia Dias:** This protocol provides a way to include in-line environmental monitoring for cells that are 'anchorage-dependent' like MSCs. That process control is an advantage over flasks that are still used in most labs or clinical production.

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

What research questions will your laboratory focus on in the future?

1.5. **Junsung Lee:** There are several open questions for MSCs and other cell types that are sensitive to mechanical cues. For example, how can this kind of approach help engineer cells to address other disease states.

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

Videographer: Obtain headshots for all authors available at the filming location.

Protocol

2. Microbioreactor Setup And Preparation

Demonstrator: Brandon Krupczak

2.1. To begin, remove the clear plastic cap connected to Bottle LL in the bottle rack **[1]**. Using a new sterile 50 milliliter syringe without a plunger, connect the nozzle to Bottle LL **[2]**. Pour the sterile filtered water into the syringe barrel and inject it into Bottle LL **[3-TXT]**.

Added shot: 2.1.0: Sterilizing bottle with ethanol

2.1.1. WIDE: Talent removing the plastic cap from Bottle LL in the rack.

2.1.2. Talent holding a 50 milliliter syringe without the plunger and connecting it to Bottle LL.

2.1.3. Talent pouring sterile filtered water into the syringe barrel and injecting it into the bottle. **TXT: Reconnect the syringe several times if the connection port resists flow**
Added shot: 2.1.3 which instructs what to do if the connection port resists flow

2.2. Similarly, fill Bottle L with 25 milliliters of low glucose DMEM without FBS, Bottle R with 25 milliliters of sterile PBS and Bottle RR with 8 milliliters of anti-adherence rinse solution **[1-TXT]**. Repeat the procedure to prepare the required number of consumables for the experiment **[2]**.

2.2.1. Shot of filled bottles L, R and RR. **TXT: Use new syringe for each solution**
Videographer's Note: Move shot as 2.2.2

2.2.2. Talent filling additional bottles using the same method with new sterile syringes.
Videographer's Note: Move shot as 2.2.1
Added shot 2.2.3:Spray with ethanol to disinfect

2.3. Next, perform a self-test on each POD *(Pod)* to be used in the experiment **[1]**. Once the POD has successfully passed all checks from the self-test, remove the self-test cassettes and place them into light-protected storage bags **[2]**. Mount the consumable to the bioreactor **[3]**.

2.3.1. Shot of PODs for testing.
AND
SCREEN: 68650_Shot1_2025-08-01-11-58-18_Step2.3.mp4 00:36-01:07
Video Editor: Please speed up the SCREEN shot

2.3.2. Talent removing cassettes from POD and placing them into light-protected bags.

2.3.3. Talent positioning the consumable near the bioreactor and preparing for

mounting.

2.4. Remove the green tape from the consumable valves **[1]** and install the consumable in the POD **[2]**. Reinstall the clamps and check for any hissing sound to confirm a secure seal **[3]**.

2.4.1. Talent peeling off green tape from the consumable valves.

2.4.2. Talent mounting the consumable in the POD.

2.4.3. Talent tightening clamps, and listening for air leaks.

2.5. Connect the white pressure sensor line, the red air pressure line, and the blue vacuum line between the consumable and the base station or POD **[1]**. Confirm that the side injection port is clamped shut using a white teardrop clamp before initiating a new experiment **[2-TXT]**.

2.5.1. Talent connecting the three colored lines from the consumable to the base station.

2.5.2. Talent inspecting and confirming the white teardrop clamp is secured on the side injection port. **TXT: Autopriming and calibration can run for 4 - 5 h or overnight**

3. Cell And Microcarrier Injection

3.1. Click the **Flush bottles** icon in the software interface **[1]**. Enable the **Radio** button for Bottle L, select **Output to Waste**, then click **Start Flush MSCserum** *(M-S-Sees-Serum)* **[2]**.
Videographer's Note: B.roll was filmed showing the device actually working based on steps filmed in the screen captures

3.1.1. SCREEN: 68650_Shot2_2025-08-01-12-12-01_Step3.1-3.3.mp4. 00:25-00:27, 00:46-00:53

3.1.2. SCREEN: 68650_Shot2_2025-08-01-12-12-01_Step3.1-3.3.mp4. 00:54-01:10

3.2. In the **Bottle Contents** window, indicate that MSCserum is located in Bottle R, which contains PBS **[1]**. Then navigate to **Manual Ops** *(opps)* and perform three eject-wash cycles on the waste bottle **[2]**.
Videographer's Note: B.roll was filmed showing the device actually working based on steps filmed in the screen captures. Footage of 3.2 can be reused in 3.3 and 3.4

3.2.1. SCREEN: 68650_Shot2_2025-08-01-12-12-01_Step3.1-3.3.mp4. 02:09-02:30

3.2.2. SCREEN: 68650_Shot2_2025-08-01-12-12-01_Step3.1-3.3.mp4 02:55-03:20

3.3. In the **Bottle Contents** window, indicate that MSCserum is now located in Bottle RR,

which contains the anti-adherence rinse solution **[1]**. Go back to **Manual Ops** and perform one eject-wash cycle on the waste bottle **[2]**.

3.3.1. SCREEN: 68650_Shot2_2025-08-01-12-12-01_Step3.1-3.3.mp4. 03:55-04:08

3.3.2. SCREEN: 68650_Shot2_2025-08-01-12-12-01_Step3.1-3.3.mp4. 04:22-04:40

3.4. After 30 minutes, rinse out the consumable with PBS **[1]**. Empty the reactor to await cell and microcarrier injection **[2].**

3.4.1. ~~Talent administering a rinse with PBS into the consumable and allowing flow-through.~~
~~**AND**~~
SCREEN:
68650_Shot3_2025-08-01-12-19-00_Step3.4.mp400:26-00:40, 00:51-00:58

3.4.2. Shot of reactor being emptied.
Videographer's Note: Reuse B.roll footage shot from 3.2

3.5. In the **Manual Ops** panel, click **Reinoculate**. Leave the system paused at the stage where the dashboard displays a prompt to inoculate the reactor **[1]**.

3.5.1. SCREEN: 68650_Shot4_2025-08-01-12-21-27_Step3.5.mp4. 00:16-00:50, -01:38-01:41

3.6. Next, pipette a calculated volume of resuspended cells into a 15-milliliter conical tube containing microcarriers for each condition **[1-TXT]**. Top up each tube to between 2.2 and 2.5 milliliters with fresh, warm human mesenchymal stem cell media **[2]**.

3.6.1. Talent pipetting cell suspension into the conical tube. **TXT: Cell volume should be enough to reach 150,000 cells**
Videographer's Note: WS and CU shots filmed

3.6.2. Talent topping up the conical tube with pre-warmed hMSC media using a pipette.

3.7. Working with one or two consumables at a time, remove them from the base station and bring them into the biosafety cabinet **[1]**. Withdraw the cell and microcarrier mixture into a 5-milliliter syringe using a green 4-inch blunt needle **[2]**. Inject the mixture into the reactor **[3]**. Then return the consumable to the base station **[4].**

3.7.1. Talent detaching consumables and transferring them carefully into the biosafety cabinet.

3.7.2. Talent drawing up the cell and microcarrier solution into the syringe.

3.7.3. Talent injecting the solution into the reactor.
Videographer's Note:
Authors thought this was a very important step that was critical to the protocol.

A and B sections were added for this step.

*Please see the author's explanation about this step in the author's notes.

*Please refer to the voice memo by the author recorded in the footage

3.7.4. Talent reconnecting the consumable to the base station.

3.8. After repeating the injection for all consumables, navigate through the software interface to indicate that inoculation is complete **[1]**. When the software returns to the home page, remove the white clamp from the consumable output lines **[2]**.

3.8.1. SCREEN: 68650_Shot5_2025-08-01-12-24-11_Step3.8.mp4 00:50-00:55, 01:06-01:08, 01:24-01:40,02:00-02:10

3.8.2. Talent removing the white clamp from the consumable output line.

3.9. Enter **Manual Ops** and perform the **Eject Excess** function with the output port set to P for perfusion **[1]**. Enable static mixing mode using a cycle of 1680 seconds on and 120 seconds off, and set mixing frequency to 5 hertz **[2]**.

3.9.1. SCREEN: 68650_Shot6_2025-08-01-12-28-25_Step3.9.mp4. 00:15-00:48

3.9.2. SCREEN: 68650_Shot6_2025-08-01-12-28-25_Step3.9.mp4. 01:53-02:47

4. Microbioreactor Harvest

4.1. To begin cell harvesting, use a 1-milliliter syringe to draw up 200 microliters of Pronase and approximately 300 to 400 microliters of air **[1-TXT]**.

4.1.1. Talent drawing 200 microliters of Pronase and then pulling 300 to 400 microliters of air into a 1 milliliter syringe. **TXT: Pronase: 10 mg/mL**

4.2. Unscrew the cap on the side port injection line **[1]** and spray the needleless valve connector with 70 percent ethanol **[2]**. Remove the tape from the knotted side port injection line, untie the knot, and unclamp the white teardrop clamp **[3]**.

4.2.1. Talent unscrewing the cap on the side port injection line.

4.2.2. Talent spraying the connector with ethanol.

4.2.3. Talent removing tape, untying the knot, and unclamping the teardrop clamp.

4.3. Now, connect the 1 milliliter syringe and gently inject the Pronase into the reactor **[1]**. Chase the liquid with air to ensure complete delivery **[2]**.

4.3.1. Talent connecting syringe and slowly injecting Pronase.
Videographer's Note: WS and CU filmed

4.3.2. Talent pressing air from the syringe to push all contents into the reactor.
Videographer's Note: WS and CU filmed

4.4. Re-engage the teardrop clamp and re-cap the injection line **[1].** Then return the consumable to the POD, reconnect all lines, remove the white C-clamp, and resume mixing **[2]**.

4.4.1. Talent securing the clamp and cap.

4.4.2. Talent reinstalling the consumable in the POD, reconnecting lines, removing C-clamp, and restarting mixing.

4.5. Place the waste bottle and media source bottle on ice **[1]**. Start a 5-minute timer and allow the Pronase to digest the microcarriers in the reactor **[2]**. After 5 minutes, verify that the microcarriers have degraded by checking their absence in the reactor **[3-TXT]**.

4.5.1. Talent positioning the bottles on ice.

4.5.2. Talent starting a 5-minute countdown.

4.5.3. Close-up view of reactor contents with no visible microcarriers. **TXT: If microcarriers are visible, allow for 1 min of additional digestion**
Videographer's Note: 2 versions filmed. Version 1 was filmed with microcarriers. Version 2 filmed with no microcarriers visible (explanation provided as audio memo in the footage)

4.6. Harvest the cells by entering **Manual Ops.** Set the number of cycles to 3, choose output port W for waste, and click on **Eject Wash [1]**.

4.6.1. SCREEN: 68650_Shot7_2025-08-01-12-36-39_Step4.6.mp4. 00:11-00:43

4.7. After the eject wash cycles are complete, transfer the consumable to the biosafety cabinet **[1].** Then withdraw the contents of the waste bottle using a 10 or 20 milliliter syringe **[2]**.

4.7.1. Shot of the consumable in a biosafety cabinet.

4.7.2. Talent drawing liquid from the waste bottle with a syringe.

4.8. Dispense the contents of the waste bottle into a 15-milliliter conical centrifuge tube **[1]**. Centrifuge the cells at 300 *g* for 5 minutes **[2]**. Aspirate the supernatant and evaluate the cell pellet size **[3]**.

4.8.1. Talent transferring waste bottle contents into a conical tube.

4.8.2. Talent placing the tube into the centrifuge and setting parameters for 300 g and 5 minutes.

4.8.3. Talent aspirating supernatant and visually inspecting the cell pellet.

Results

5. Results

5.1. Environmental conditions including pH, temperature, dissolved oxygen, and carbon dioxide were stably maintained throughout the 10-day culture period in the microbioreactor system **[1]**. The pH variation was significantly lower in the microbioreactor compared to the tissue culture polystyrene flask condition **[2]**.

5.1.1. LAB MEDIA: Figure 2A-D *Video Editor: please sequentially show A to D*

5.1.2. LAB MEDIA: Figure 2E. *Video editor: Please highlight "Breez" boxplot.*

5.2. Cell yield was higher in the microbioreactor than in the T25 *(T-Twenty-Five)* flask, while maintaining similar cell viability **[1]**.

5.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the bar for "GMC Breez"*

5.3. Of the 14 measured potency-associated critical quality attributes **[1]**, 9 showed significantly higher mRNA expression in the microbioreactor condition relative to the T75 flask **[2]**.

5.3.1. LAB MEDIA: Figure 4. *Video editor: Show the bottom graph*

5.3.2. LAB MEDIA: Figure 4. *Video editor: Show the zoomed out upper graph*

Pronunciation Guide:

1. Microbioreactor

- **Pronunciation link:**
- **IPA (American):** /ˌmaɪkroʊˌbaɪ.oʊˈriːæk.tər/
- **Phonetic Spelling:** my-kroh-bye-oh-REE-ak-ter

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2. Mesenchymal

- **Pronunciation link:**
- **IPA (American):** /mɛsˈɛn.kɪ.məl/
- **Phonetic Spelling:** mes-EN-ki-muhl

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3. Mesenchymal stromal cells

- **Pronunciation link:** via HowToPronounce.com (audio available) YouGlish+6How To Pronounce+6YouTube+6
- **IPA (American):** /mɛsˈɛn.kɪ.məl ˈstroʊ.məl sɛlz/
- **Phonetic Spelling:** mes-EN-ki-muhl STROH-muhl sells

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4. Anchorage-dependent

- **Pronunciation link:** No confirmed link found
- **IPA (American):** /ˈæŋ.kər.ɪdʒ-dɪˈpɛn.dənt/
- **Phonetic Spelling:** ANG-ker-ij dih-PEN-dent

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5. Stromal

- **Pronunciation link:** implicit in Collins; pronunciation follows "Mesenchymal stromal cells" above YouTube+11Collins Dictionary+11How To Pronounce+11
- **IPA (American):** /ˈstroʊ.məl/
- **Phonetic Spelling:** STROH-muhl

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6. Pronase

- **Pronunciation link:** No confirmed link found
- **IPA (American):** /ˈproʊ.neɪs/
- **Phonetic Spelling:** PROH-nayss

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7. Perfusion

- **Pronunciation link:** No confirmed link found
- **IPA (American):** /pərˈfjuː.ʒən/
- **Phonetic Spelling:** per-FYOO-zhun

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8. Eject-wash

- **Pronunciation link:** No confirmed link found (composite of eject + wash)
- **IPA (American):** /iˈdʒɛkt wɔʃ/
- **Phonetic Spelling:** ee-JEKT awsh

9. Inoculation

- **Pronunciation link:** No confirmed link found
- **IPA (American):** /ɪˌnɑː.kjəˈleɪ.ʃən/
- **Phonetic Spelling:** ih-NAH-kyoo-LAY-shun

10. Culture polystyrene flask

- **Pronunciation link:** No confirmed link found for full phrase; “polystyrene” separately
- **IPA (American):**
  - *polystyrene*: /ˌpɑː.liˈstaɪ.riːn/
  - *flask*: /flæsk/
- **Phonetic Spelling:** pah-lee-STY-reen flask