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Title: A Platform for the Continuous Culture of Endothelial Cells under Physiological Flow Conditions in Simulated Microgravity

Authors and Affiliations:

Joshua Tran¹, Charmaine Lui², Max Kim¹, Sang-Joon John Lee², Anand K. Ramasubramanian¹

¹Department of Chemical and Materials Engineering, San José State University

²Department of Mechanical Engineering, San José State University

Corresponding Authors:

Anand Ramasubramanian anand.ramasubramanian@sjsu.edu

Email Addresses for All Authors:

joshua.tran@sjsu.edu

charmaine.lui@sjsu.edu

max.kim@sjsu.edu

sang-joon.lee@sjsu.edu

anand.ramasubramanian@sjsu.edu

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

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes.**

Current Protocol Length

Number of Steps: 21

Number of Shots: 47

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Joshua Tran:** We develop microfluidic systems for continuous endothelial culture to investigate how simulated microgravity and physiological flow affect cellular function.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Joshua Tran:** Existing systems struggle with bubbles and combined microgravity–venous flow studies; this platform enables bubble-free continuous culture under both conditions.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Joshua Tran:** This protocol enables researchers to measure fluid-induced shear stress and assess changes in cell morphology under simulated microgravity.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Joshua Tran:** The most challenging step is priming the system, which requires considerable manual dexterity to establish a sterile, bubble-free flow loop.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Joshua Tran:** Future studies can explore system modifications to increase shear stress output and use transcriptomic analyses to characterize cellular responses to microgravity and flow.
 - 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What is the key takeaway you hope viewers remember after watching your video article?

- 1.6. **Joshua Tran, Graduate Research Assistant:** (authors will preset their testimonial statements live)

What value do you expect this video article to have for other researchers working in your field?

- 1.7. **Joshua Tran, Graduate Research Assistant:** (authors will present their testimonial statements live)

How does the video format of your article help you to share your work with the broader scientific community?

- 1.8. **Joshua Tran, Graduate Research Assistant:** (authors will present their testimonial statements live)

Protocol

Videographer: Please always record an audio slate while filming each shot mentioned in the shotlist.

2. System Electronics

Demonstrator: Joshua Tran

2.1. Begin by connecting the pump driver and pump cable to the microcontroller and attaching the micropump to the pump cable [1]. Connect the Nano board and liquid flow sensor to a computer containing their respective applications [2].

2.1.1. WIDE: Talent connecting the pump driver, pump cable, and micropump to the microcontroller.

2.1.2. Talent connecting the Nano board and the liquid flow sensor to the computer.

2.2. Before starting sterile preparation, connect the mobile computer station to the microcontroller using the mini-USB cable, keeping the USB and pump leads accessible outside the protective bag [1]. Open the microcontroller application, set both micropumps to 100 hertz and 240 volts, while keeping the pumps off [2].

2.2.1. Talent arranging the USB and pump leads to easy access to the mobile computer before enclosing the microcontroller.

2.2.2. CU: Shot of the microcontroller application showing both pumps being set to 100 hertz and 240 volts.

3. System Fluidics Connections

3.1. Prepare four 10-centimeter lengths of PVC (*p-v-c*) tubing for the connections [1]. Leave the bubble trap empty during dry assembly [2-TXT]. Seal the bubble trap hermetically to ensure a closed loop system during sterilization and priming [3]. **NOTE:** The VO is edited for the additional shot

3.1.1. Talent cutting the PVC tubing to various measured lengths needed for the flow loop.

3.1.2. Talent leaving the bubble-trap cavity empty during dry assembly. **TXT: Fill the bubble trap ~80% with working fluid during priming**

3.1.3. **Talent sealing the bubble trap top port with Teflon tape and a Luer plug.** **NOTE:** Additional shot

- 3.2. Slide a 3-millimeter black nylon round spacer over the connection to secure it [1]. Connect one prepared PVC tube to the micropump outlet, following the arrow that indicates the flow direction [2], and connect the other end of the tube to either side port of the bubble trap [3]. **NOTE: The VO is swapped for the inverted shots**
- 3.2.2 Talent securing the tubing to the micropump with the 3-millimeter black nylon round spacer.
- 3.2.1. Talent attaching the PVC tubing after identifying the micropump outlet from the flow-direction arrow.
- ~~Talent securing the tubing to the micropump with the 3-millimeter black nylon round spacer.~~ **NOTE: 3.2.1 and 3.2.2 should be swapped**
- 3.2.2. Talent connecting the free tubing end to one side port of the bubble trap.
- 3.3. Use the second PVC tube to connect the other side port of the bubble-trap to the inlet barb of the liquid flow sensor [1]. Connect the third tube from the sensor outlet, on the side toward which the arrow points, to an elbow Luer connector [2].
- 3.3.1. Talent connecting the bubble-trap outlet to the liquid flow sensor inlet with a 25-centimeter PVC tubing.
- 3.3.2.** Talent connecting the flow sensor outlet to an elbow Luer connector with the second 25-centimeter tube. **Videographer notes: There's an elbow on each side. I created a shot slated 3.3.3 that is a tight shot of the elbow getting attached.]**
- 3.4. Then, connect the fourth and final PVC tube to the micropump inlet and secure it with a 3-millimeter black nylon round spacer as demonstrated [1]. Connect its free end to the second elbow Luer connector [2] to complete the flow-loop tubing assembly [3].
- 3.4.1. WIDE: Shot of PVC tubing being connected to the micropump inlet and then securing it with a black nylon round spacer.
- 3.4.2. Talent connecting the free end of the PVC tube to a second elbow Luer connector.
- 3.4.3. WIDE: Shot of completed flow loop showing the pump, bubble trap, flow sensor, and two elbow Luer connectors in the correct flow direction.

4. Experimental Preparation and System Sterilization **Demonstrator: Charmaine Lui**

- 4.1. Prepare a laminar flow hood for system sterilization [1]. Fill one 50-milliliter self-standing centrifuge tube with 70% ethanol and a second with sterile cell culture water [2].
 - 4.1.1. WIDE: Talent preparing the biosafety cabinet for the sterilization procedure.
Videographer notes: Talent couldn't get a biosafety cabinet for the filming. So they're using a hood, which is similar. All of these shots are tight.]
 - 4.1.2. Talent filling labeled 50-milliliter tubes with 70% ethanol and sterile cell-culture water, respectively.
- 4.2. Place the microcontroller inside a small plastic bag, while leaving the pump cable, micropump, and connected mini-USB lead outside [1]. ~~Seal the bag with a zip tie [1].~~ Wipe the exterior of all flow-loop components and experimental materials with 70% ethanol before transferring them into the biosafety cabinet [2]. NOTE: The VO is edited for the deleted shot
 - 4.2.1. Talent enclosing the microcontroller in a plastic bag, while leaving the pump cable, micropump, and mini-USB lead outside the bag. NOTE: This is a repeat of 2.2.1
 - 4.2.2. ~~Talent sealing the plastic bag containing the microcontroller with a zip tie.~~ NOTE: Not filmed
 - 4.2.3. Talent disinfecting the flow-loop components before placing them into the biosafety cabinet.
- 4.3. Connect a 12-milliliter syringe to the other elbow connector in the same loop using a female-to-female Luer coupler [1]. Submerge the elbow Luer connector attached to the micropump in the 50-milliliter tube of 70% ethanol [2]. NOTE: The VO is swapped for the inverted shots
 - 4.3.2 Talent attaching the 12-milliliter syringe to the outlet elbow connector with the female-to-female Luer coupler.
 - 4.3.1. Talent submerging the inlet elbow Luer connector in 70% ethanol.
 - 4.3.2. ~~Talent attaching the 12-milliliter syringe to the outlet elbow connector with the female-to-female Luer coupler.~~ Videographer notes: These need to be swapped. Talent has to hold the elbow in the ethanol with one hand during pumping]

- 4.4. Pinch the PVC tubing between the micropump and elbow connector and confirm that the bubble-trap top port is sealed with a Luer plug [1]. Pull the syringe plunger approximately 5 milliliters to create a vacuum throughout the system [2].
 - 4.4.1. Talent pinching the PVC tubing to check that the bubble-trap top port is sealed with a Luer plug.
 - 4.4.2. Talent pulling the syringe plunger approximately 5 milliliters to create a vacuum throughout the system.
- 4.5. Power on the micropump, release the pinched tubing, and allow 5 milliliters of 70% ethanol to pass through the loop into the syringe [1]. Open the bubble-trap top port and fill the cavity to approximately 80% with ethanol [2]. Then, turn off the pump, reseal the port [3], and gently shake the trap to sterilize its interior [4].
 - 4.5.1. Talent switching on the micropump, after releasing the pinched tubing to let 5 milliliters of ethanol through the loop.
 - 4.5.2. Talent opening the bubble-trap top port to fill the cavity to approximately 80% with ethanol.
 - 4.5.3. WIDE: Shot of micropump being turned off, bubble-trap top port being resealed.
 - 4.5.4. Talent gently agitating the trap to expose all internal surfaces to the 70% ethanol.
- 4.6. Remove the inlet elbow connector from the ethanol, then run the micropump until the ethanol enters the syringe [1]. Disconnect and empty the bubble trap into an appropriate waste container [2]. Reassemble the loop and repeat the flushing procedure using sterile cell-culture water, passing 15 milliliters through each flow loop [3].
 - 4.6.1. Talent removing the inlet elbow connector from ethanol while running the pump to remove the residual ethanol from the loop.
 - 4.6.2. Talent empties the bubble trap into the designated ethanol waste container. Videographer notes: I've included four different versions of this shot. V3 is best for showing the full system during emptying. V1 gives a clearer shot of the syringe being emptied. V4 is a tight shot of the bubble trap being emptied, it also is the only version where the teflon tape stays on successfully.]

4.6.3. WIDE: Shot of flushing procedure being repeated with sterile cell-culture water.

5. System Priming and Microfluidic Cell-Channel Integration

5.1. After sterilization, keep the system in the laminar flow hood for vacuum priming [1]. Reconnect the 12-milliliter syringe to the outlet elbow connector [2] and submerge the inlet elbow connector in a container with 30 milliliters of cell-culture medium [3].

NOTE: The VO is edited for the swapped shots

5.1.1. WIDE: The sterilized system left inside the biosafety cabinet for vacuum priming.

5.1.3 Talent connecting the 12-milliliter syringe to the outlet elbow. **NOTE: 5.1.2 and 5.1.3 are swapped**

5.1.2. Talent submerging the inlet elbow in cell-culture medium.

5.2. Clamp the PVC tubing between the micropump and elbow connector, verify that the bubble trap is sealed, and pull the syringe plunger approximately 5 milliliters to establish a vacuum [1]. Start the micropump after releasing the clamp and pass 5 milliliters of cell-culture medium through the loop [2].

5.2.1. Shot of tubing being clamped and the seal on the bubble trap being verified, before pulling the syringe plunger to establish the vacuum.

5.2.2. Talent starting the pump, after releasing the clamp, to draw 5 milliliters of cell-culture medium through the loop.

5.3. Open the top port of the bubble trap and allow it to fill to approximately 80% with cell-culture medium [1]. Turn off the pump and reseal the top port [2].

5.3.1. Talent filling the bubble trap to approximately 80% with cell-culture medium.

5.3.2. Talent switching off the pump, bubble trap top port being resealed.

5.3.3. ~~Talent sealing the bubble trap top port with Teflon tape and a Luer plug after.~~
NOTE: This is Moved to 3.1.3.

5.4. Place the endothelial-cell microfluidic slide, cultured to approximately 80% confluence, fully submerged in the medium-filled container [1], and position a magnetic stir bar at its center to prevent it from floating [2].

5.4.1. CU: Talent placing the microfluidic slide with cultured endothelial cells in the medium filled container.

5.4.2. Talent positioning a magnetic stir bar on the slide to prevent floating.

5.5. Keeping both elbow connectors submerged, run the micropump while connecting them to the microchannels [1]. Confirm that no bubbles form at either junction before finally turning off the pump [2].

5.5.1. Talent connecting both, elbow Luer connectors to the microchannel under the medium layer while the micropump is running.

5.5.2. Shot of the micropump being turned off after the completely submerged connectors show no visible bubbles at either junction.

6. Final System Assembly and Operation

6.1. ~~Complete the upper deck assembly by mounting the flow sensors and bubble traps above the enclosure, supporting the deck with threaded rods and spacers, and securing the microfluidic slide and its connectors between the two levels [1].~~ Fasten the assembled flow system to the random positioning machine, with the microfluidic slide centered on the rotational axes, and secure all components and cables without stressing the electrical connections [1]. **NOTE: The VO is edited for the deleted shot**

6.1.1. ~~WIDE: Shot of upper deck being assembled with flow sensor clamps, bubble trap holders, and cushioned microfluidic slide mount.~~ **NOTE: Not filmed**

6.1.2. WIDE: Shot of mounting the completed system on the random positioning machine and securely placing the microfluidic slide centered on the rotational axes.

6.2. Connect the RPM, microcontroller, and liquid flow sensors to the computer and power supplies [1]. Open the flow-sensor software, adjust the pump voltage to reach the desired flow rate, and verify bubble-free, leak-free flow and unobstructed rotation for 1 hour [2]. Then, begin logging flow data for the 24-hour experimental period [3].

6.2.1. Talent connecting the assembled system to the computer and power supplies.

NOTE: Videographer notes: Included two versions of system being plugged in. V1 is just the pump. V2 is the everything after talent figured out how to secure the cords on the RPM]

6.2.2. WIDE : Shot of the running system being inspected for leaks and bubbles, while simultaneously verifying that the RPM control rotates freely.

6.2.3. Talent starting the 24-hour flow-data log at the target flow rate.

Results

7. Results

- 7.1. Representative flow-rate plots showed substantial fluctuations and frequent sharp spikes in the flow loop without the orientation-independent bubble trap [1]. In contrast, the loop containing the bubble trap maintained a more stable flow rate with only occasional minor deviations [2].
- 7.1.1. LAB MEDIA: Figure 4 *Video Editor: Please highlight the **left plot** and use arrows to emphasize the **tall, irregular blue spikes**.*
- 7.1.2. LAB MEDIA: Figure 4 *Video Editor: Please highlight the **right plot** and use arrows to emphasize the **narrow, stable blue band**.*
- 7.2. Following the microgravity experiment, extensive endothelial-cell detachment was observed in the channel without a bubble trap [1]. In contrast, the bubble-trap condition retained a dense layer of adherent cells with clearly preserved actin structures and nuclei, indicating rescue of cell viability and growth [2].
- 7.2.1. LAB MEDIA: Figure 5 *Video Editor: Please highlight in Figure 5A, the **sparse or empty areas** and use arrows to highlight the **detached or remaining isolated cells**.*
- 7.2.2. LAB MEDIA: Figure 5 *Video Editor: Please highlight in Figure 5B, the **dense fluorescent cell layer** and use arrows to emphasize the **intact red actin structures and blue-stained nuclei**.*

1. Microcontroller
Pronunciation link: <https://www.merriam-webster.com/dictionary/microcontroller>
IPA: /ˌmaɪkroʊkənˈtroʊlər/
Phonetic Spelling: my·kroh·kuhn·TROH·ler
2. Micropump
Pronunciation link: No confirmed link found
IPA: /ˈmaɪkroʊˌpʌmp/
Phonetic Spelling: MY·kroh·pump
3. Hermetically
Pronunciation link: <https://www.merriam-webster.com/dictionary/hermetically>
IPA: /ˌhɜːˈmɛtɪkli/
Phonetic Spelling: her·MET·ik·lee
4. Sterilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilization>
IPA: /ˌstɛrələˈzeɪʃən/
Phonetic Spelling: stair·uh·luh·ZAY·shuhn
5. Priming
Pronunciation link: <https://www.merriam-webster.com/dictionary/priming>
IPA: /ˈpraɪmɪŋ/
Phonetic Spelling: PRY·ming
6. Nylon
Pronunciation link: <https://www.merriam-webster.com/dictionary/nylon>
IPA: /ˈnaɪˌlɑːn/
Phonetic Spelling: NY·lahn
7. Luer
Pronunciation link: No confirmed link found
IPA: /ˈluːər/
Phonetic Spelling: LOO·er
8. Laminar
Pronunciation link: <https://www.merriam-webster.com/dictionary/laminar>
IPA: /ˈlæmənər/
Phonetic Spelling: LAM·uh·ner
9. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: SEN·truh·fyooj
10. Biosafety
Pronunciation link: No confirmed link found
IPA: /ˌbaɪoʊˈseɪfti/
Phonetic Spelling: by·oh·SAYF·tee
11. Microfluidic
Pronunciation link: No confirmed link found
IPA: /ˌmaɪkroʊfluˈɪdɪk/
Phonetic Spelling: my·kroh·floo·ID·ik

12. Endothelial
 Pronunciation link: <https://www.merriam-webster.com/medical/endothelial>
 IPA: /,ɛndəʊˈθiːliəl/, /,ɛndəˈθiːliəl/
 Phonetic Spelling: en·doh·THEE·lee·uhl / en·duh·THEE·lee·uhl
13. Confluence
 Pronunciation link: <https://www.merriam-webster.com/dictionary/confluence>
 IPA: /ˈkɑːnfluəns/
 Phonetic Spelling: KON·floo·uhns
14. Microchannels
 Pronunciation link: No confirmed link found
 IPA: /ˈmaɪkroʊˌtʃænəlz/
 Phonetic Spelling: MY·kroh·chan·uhlz
15. Microgravity
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microgravity>
 IPA: /,maɪkroʊˈgrævəti/
 Phonetic Spelling: my·kroh·GRAV·uh·tee
16. Orientation-independent
 Pronunciation link: No confirmed link found
 IPA: /,ɔːriənˈteɪʃən ˌɪndɪˈpɛndənt/
 Phonetic Spelling: or·ee·uhn·TAY·shuhn in·dih·PEN·duhnt
17. Fluctuations
 Pronunciation link: <https://www.merriam-webster.com/dictionary/fluctuation>
 IPA: /ˌflʌktʃuˈeɪʃənz/
 Phonetic Spelling: fluk·choo·AY·shuhnz
18. Endothelial-cell
 Pronunciation link: <https://www.merriam-webster.com/medical/endothelial>
 IPA: /,ɛndəʊˈθiːliəl sɛl/, /,ɛndəˈθiːliəl sɛl/
 Phonetic Spelling: en·doh·THEE·lee·uhl sell / en·duh·THEE·lee·uhl sell
19. Viability
 Pronunciation link: <https://www.merriam-webster.com/dictionary/viability>
 IPA: /ˌvaɪəˈbɪləti/
 Phonetic Spelling: vy·uh·BIL·uh·tee
20. Nuclei
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclei>
 IPA: /ˈnuːkliˌaɪ/
 Phonetic Spelling: NOO·klee·eye