Submission ID #: 68722
Scriptwriter Name: Pallavi Sharma
Project Page Link: https://review.jove.com/account/file-uploader?src=20960258

Title: An Open-Source Normothermic Perfusion System Designed for Research Scientists

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

☐ Incorrect

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

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

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **Already completed and uploaded video files**

Authors: Since we have protocol footage, this question is applicable for filming interview statements

When you are ready to submit your video files, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 27
Number of Shots: 55

Introduction

1.1. **Jonathan Hernandez:** The machine allows comprehensive interrogation of tumor-bearing organs by recapitulating the complex tumor-tissue microenvironment and pathophysiological processes, enabling real-time investigation of tumor biology, therapeutic response, and microenvironment interactions.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: Figure 1*

What research gap are you addressing with your protocol?

1.2. **Jonathan Hernandez:** The machine presented herein addresses the lack of affordable normothermic perfusion systems for research applications, enabling iterative studies where current commercial machines are limited by cost and inflexibility.

1.2.1. 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?

1.3. **Jonathan Hernandez:** Our laboratory will continue working to further scale the machine down to enable completely autologous perfusion for immune-oncology investigations and therapeutics.

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

Ethics Title Card

This research is performed in accordance with the ethical standards of our institution's Human Research Ethics Committee and adheres to all applicable guidelines and regulations for research involving human subjects and tissues

Protocol

2. Machine Set-Up Normothermic Ex Vivo Perfusion System

Demonstrator: Lindsay R Friedman

NOTE: The script protocol section is drafted from available footage

2.1. To begin, place the drum funnel on the right side of the workstation with the spout extending past the front edge of the table **[1]**. Place a circular memory foam to fit the base of the drum, with the diaphragm positioned underneath the foam **[2].** Leave a 4-inch gap near the spout for outflow **[3]**.

2.1.1. LAB MEDIA: 1.1.4.MOV: 00:003-00:06

2.1.2. LAB MEDIA: 1.1.4.MOV: 00:07-00:12

2.1.3. LAB MEDIA: 1.1.4.MOV: 00:13-00:16

2.2. Then, place two water heating blankets on top of the foam insert with the connector tubing facing the top left corner and insert a three-sixteenth inch male Luer barb into each tube **[1]**.

2.2.1. LAB MEDIA: 1.1.5.MOV: 00:02-00:13

2.3. Take a folded sterile warmer drape, cut 2 millimeters off the corner of the fold to create an outflow hole, and place it on the drum **[1]**.

2.3.1. LAB MEDIA: 1.1.6.MOV: 00:02-00:09, 00:23-00:25

2.4. Next, obtain the 3D-printed outlet connector and insert the magnet into its groove **[1]**. Place this assembly beneath the drape through the outflow spout and align the cut hole with the hole in the connector and the magnet **[2]**.

2.4.1. LAB MEDIA: 1.1.7.MOV: 00:03-00:06

2.4.2. LAB MEDIA: 1.1.7.MOV: 00:10-00:15, 00:20-00:25

2.5. Raise the excess drape to cover the drum funnel and secure it using rubber bands or elastic ties **[1]**. Fold the excess drape over the top to establish a sterile barrier **[2]**. Now, place the second water jacket on top of the folded drape **[3]**. Then, take a 30-inch piece of one-quarter inch tubing and connect the proximal end to the outlet at the base of the drum funnel spout and rest near the reservoir holder, which will be connected later **[4]**.

2.5.1. LAB MEDIA: 1.1.8.MOV: 00:07-00:14.

2.5.2. LAB MEDIA: 1.1.8.MOV: 00:17-00:23

2.5.3. LAB MEDIA: 1.1.8.MOV: 00:26-00:29

2.5.4. LAB MEDIA: 1.1.8.MOV: 00:05-00:16.

2.6. Now, place the pumps on a mobile cart positioned near the left side of the workstation **[1]**. Position the stand and two clamps directly behind the pumps so the clamps are elevated 6 inches above the pumps **[2]**.

2.6.1. LAB MEDIA: 1.2.1.MOV: 00:02-00:06

2.6.2. LAB MEDIA: 1.2.1.MOV: 00:08-00:13.

2.7. Further, obtain two pump heads and secure each into one of the pumps **[1]**. Place two oxygenators into the clamps on the stand in a horizontal orientation **[2]**. Then, connect the arterial and venous one-eighth inch gas inflow tubing to the top right Luer connector of their respective oxygenators **[3]**.

2.7.1. LAB MEDIA: 1.2.2.MOV: 00:04-00:11

2.7.2. LAB MEDIA: 1.2.3.MOV: 00:03-00:13

2.7.3. LAB MEDIA: 1.3.3.MOV: 00:05-00:13

2.8. Now, remove all tubing from the hemodialysis kit except for the lines leading directly into the filter **[1]**.

2.8.1. LAB MEDIA: 1.4.1.MOV: 00:05-00:14

2.9. Place one large roller pump immediately to the right of the hemodialysis filter for blood inflow **[1]**. Then, place two smaller peristaltic pumps to the right of the blood roller pump for dialysate inflow and outflow **[2]**. Obtain two one-eighth-inch peristaltic tubings and insert them into the white pump clamp. Attach a female one-eighth-inch Luer barb to both ends of each tubing **[3]**.

2.9.1. LAB MEDIA: 1.4.2.MOV: 00:03-00:04

2.9.2. LAB MEDIA: 1.4.2.MOV: 00:05-00:07.

2.9.3. LAB MEDIA: 1.4.2.1.MOV: 00:05-00:14

2.10. To connect the dialysate bag to the filter inflow, attach one-eighth inch tubing to the Luer connector on the bag **[1]**. Attach a male one-eighth inch Luer to the tubing's end and connect it to the inflow peristaltic pump tubing **[2]**. Connect inflow and outflow circuits to the appropriate hemodialysis filter ports **[3].** Place the reservoir inside the reservoir holder with the exterior measurement markings and the yellow-capped connector facing outward **[4]**.

2.10.1. LAB MEDIA: 1.4.3.1.MOV: 00:03-00:10

2.10.2. LAB MEDIA: 1.4.3.1.MOV: 00:10-00:14

2.10.3. LAB MEDIA: 1.4.3.1.still.JPEG

2.10.4. LAB MEDIA: 1.5.1.MOV: 00:04-00:10.

2.11. Next, cut a 36-inch piece of five-sixteenth-inch tubing, attach a Y connector to the distal end of this tubing, and connect the proximal end to the outflow spigot at the bottom of the reservoir **[1]**.

2.11.1. LAB MEDIA: 1.5.2.MOV: 00:08-00: 18

2.12. Cut two 4-inch pieces of five-sixteenth inch tubing and attach each to the end of the Y connector **[1]**. Connect the other ends of these tubes to the horizontal prongs of the pump heads **[2]**.

2.12.1. LAB MEDIA: 1.5.3.MOV: 00:03-00:17

2.12.2. LAB MEDIA: 1.5.3.MOV: 00:19-00:24

2.13. Then, cut two 4-inch pieces of one-quarter inch tubing and connect one to each vertical barb on the pump heads. Attach the other end to the left lower prong of each oxygenator **[1]**.

2.13.1. LAB MEDIA: 1.5.4.MOV: 00:03-00:20

2.14. Now, take a 30-inch segment of five-sixteenth inch tubing, cut the tubing in half, and place a five-sixteenth inch Y connector over the distal cut end **[1]**. Attach the proximal end to the outflow barb of the venous oxygenator. Reattach the other half to the top barb of the Y connector **[2].**

2.14.1. LAB MEDIA: 1.6.1.MOV: 00:02-00:05, 00:12-00:15

2.14.2. LAB MEDIA: 1.6.1.+.MOV: 00:19-00:21

2.15. For the side sampling circuit, cut a 2-inch piece of one-quarter inch tubing and, using a needle driver, stretch the proximal end **[1]**. Attach this stretched tubing to the remaining barb of the five-sixteenth inch Y connector **[2]**.

2.15.1. LAB MEDIA: 1.6.2.MOV: 00:02-00:07

2.15.2. LAB MEDIA: 1.6.2.MOV: 00:12-00:16.

2.16. After connecting the cuvette shunt sensor, connect the horizontal part of a female T connector to a one-eighth inch tubing fitted with a male Luer**.** Attach a sampling clave to the perpendicular aspect of the T connector **[1]**. Finally, connect a one-eighth inch male Luer to the other horizontal side of the T connector **[2]**.

2.16.1. LAB MEDIA: 1.6.2.3. MOV: 00:03-00:13

2.16.2. LAB MEDIA: 1.6.2.3. MOV: 00:14-00:20

2.17. Then, cut a 12-inch length of one-eighth inch tubing and connect the proximal end to the sampling clave **[1]**. Attach a male one-eighth inch Luer to the distal end and connect it to a second female T connector **[2]**. To one side of this T connector, attach a one-quarter-inch male Luer and use a 2-inch piece of one-quarter inch tubing to connect it to a reservoir inflow barb **[3]**.

2.17.1. LAB MEDIA: 1.6.2.3. MOV: 00:23-00:25

2.17.2. LAB MEDIA: 1.6.2.3. MOV: 00:27-00:31

2.17.3. LAB MEDIA: 1.6.2.3. MOV: 00:32-00:37

2.18. At the distal end of the five-sixteenth inch tubing, attach another five-sixteenth inch Y connector **[1]**. Obtain approximately 55 inches of one-quarter inch tubing and connect it to the top barb of the Y connector **[2]**.

2.18.1. LAB MEDIA: 1.6.3.MOV: 00:03-00:08

2.18.2. LAB MEDIA: 1.6.3.MOV: 00:14-00:23

2.19. For the hemodialysis blood circuit, attach a one-quarter-inch male Luer to the distal end of the one-quarter inch tubing **[1]**. Thread this tubing through the large roller pump **[2]** and connect it to the blood inflow port at the top of the hemodialysis filter, identified by red-lined tubing **[3]**.

2.19.1. LAB MEDIA: 1.6.4.MOV: 00:02-00:11

2.19.2. LAB MEDIA: 1.6.4.MOV: 00:12-00:19

2.19.3. LAB MEDIA: 1.6.4.MOV: 00:26-00:31

2.20. Next, take a 24-inch piece of one-quarter inch tubing and attach a male Luer to the proximal end **[1]**. Connect this tubing to the blue-lined blood outflow port of the hemodialysis filter. Then, attach the distal end to the inflow barb of the reservoir **[2]**.

2.20.1. LAB MEDIA: 1.6.4.1.MOV: 00:01-00:09

2.20.2. 1.6.4.1.MOV: 00:10-00:18

2.21. Take a 36-inch piece of one-quarter inch tubing and stretch it to fit securely onto the barb of the five-sixteenth inch Y connector **[1]**.

2.21.1. LAB MEDIA: 1.6.5.MOV: 00:01-00:12.

2.22. Next, obtain three four-way stopcocks and connect them in a linear sequence **[1]**. Attach this assembly to the perpendicular port of a female T connector **[2]**. Insert this component 12 inches from the left side of the one-quarter inch tubing **[3]**.

2.22.1. LAB MEDIA: 1.6.6.MOV: 00:02-00:05

2.22.2. LAB MEDIA: 1.6.6.MOV: 00:06-00:12

2.22.3. LAB MEDIA: 1.6.6.MOV: 00:13-00:18

2.23. Then, attach external flow sensors to the one-quarter inch tubing immediately downstream of the drug infusion site **[1]**. Make a cut 2 inches distal to the flow sensors and insert pressure sensors with barbed ends into the one-quarter inch tubing. Place the distal portion of the tubing into the center of the drum funnel **[2]**.

2.23.1. LAB MEDIA: 1.6.7.MOV: 00:04-00:12

2.23.2. LAB MEDIA: 1.6.7.part2MOV:00:07-00:20

2.24. Next, cut a 30-inch length of five-sixteenth inch tubing and connect its proximal end to the right outflow barb of the arterial oxygenator **[1]**. Cut this tubing at the midpoint and insert a five-sixteenth inch Y connector **[2-TXT]**.

2.24.1. LAB MEDIA: 1.7.1.MOV: 00:05-00:10

2.24.2. LAB MEDIA: 1.7.1.MOV: 00:13-00:25 **TXT: Create a duplicate side sampling circuit**

2.25. Then, cut a 36-inch segment of one-quarter inch tubing and connect it to the barb of the five-sixteenth inch Y connector **[1]**. Reattach the external flow sensor, make a cut 2 inches downstream, and connect pressure sensors to the tubing end positioned at the drum funnel center for arterial inflow. Then, cut a 30-inch piece of quarter-inch tubing and attach a quarter-inch male Luer to the proximal end for eventual connection to the specimen's inferior vena cava outflow **[2]**.

2.25.1. LAB MEDIA: 1.7.3.MOV: 00:01-00:10

2.25.2. LAB MEDIA: 1.8.1.MOV: 00:02-00:09

2.26. Cut this tubing 6 inches from the reservoir and attach one-quarter-inch male Luer to both ends. Insert a female T connector with a sampling clave on the perpendicular port **[1]**.

2.26.1. LAB MEDIA: 1.8.2.MOV and 1.8.2.final.machine.jpeg

2.27. Introduce perfusate into the circuit following the previously described method. Rewarm and connect the specimen and begin perfusion **[1]**.

2.27.1. LAB MEDIA: 1.9.MOV: 00:11-00:25

Results

3. Results

3.1. In the first 24 hours of porcine liver perfusion, hepatic artery and portal vein flows averaged 107 and 501 milliliters per minute **[1],** respectively, with stable inflow pressures of 80 and 7.1 millimeters of mercury **[2].**

3.1.1. LAB MEDIA: Figure 2A.

3.1.2. LAB MEDIA: Figure 2B.

3.2. Arterial, portal, and venous oxygen saturations in porcine livers averaged 95%, 85%, and 74% **[1],** with pH remaining near 7.2 **[2].**

3.2.1. LAB MEDIA: Figure 2C.

3.2.2. LAB MEDIA: Figure 2D.

3.3. Glucose levels decreased steadily **[1],** hematocrit and hemoglobin remained stable **[2],** and potassium dropped slightly **[3].** Porcine livers produced 41 milliliters of bile **[4]** and showed moderate lactate elevation **[5].**

3.3.1. LAB MEDIA: Figure 2F.

3.3.2. LAB MEDIA: Figure 2E**.** *Video editor: Show the top panel for hematocrit*

3.3.3. LAB MEDIA: Figure 2E**.** *Video editor: Show the bottom panels for hematocrit and potassium*

3.3.4. LAB MEDIA: Figure 2H.

3.3.5. LAB MEDIA: Figure 2G.

3.4. In human perfusion, arterial and venous inflows averaged 113 and 317 milliliters per minute **[1],** and oxygen saturation reached 98%, 92%, and 86% in arterial, portal, and hepatic veins **[2].**

3.4.1. LAB MEDIA: Figure 3A.

3.4.2. LAB MEDIA: Figure 3C.

3.5. Human livers maintained physiologic pH **[1],** stable hematocrit and hemoglobin **[2],** and showed glucose decline and lactate increase **[3],** with bile production averaging 1.4 milliliters per hour **[4].**

3.5.1. LAB MEDIA: Figure 3D.

3.5.2. LAB MEDIA: Figure 3I.

3.5.3. LAB MEDIA: Figure 3F–G.

3.5.4. LAB MEDIA: Figure 3H.

3.6. Histology confirmed viable hepatic parenchyma and tumor morphology after perfusion **[1].**

3.6.1. LAB MEDIA: Figure 3J–L.

3.7. A mobile setup enabled CT *(C-T)* imaging of perfused livers, yielding radiographic quality comparable to in vivo scans **[1].** The system also supported kidney and pancreas perfusion with preserved glomeruli and Islets of Langerhans **[2].**

3.7.1. LAB MEDIA: Figure 4B.

3.7.2. LAB MEDIA: Figure 4C–F.

Pronunciation Guide

1. Diaphragm

- **Pronunciation link** (American English): https://www.merriam-webster.com/dictionary/diaphragm
- **IPA**: /ˈdaɪ-ə-fræm/ (How To Pronounce)
- **Phonetic Spelling**: *dye-uh-fram*

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2. Hemodialysis

- **Pronunciation link**: (YouTube tutorial) https://www.youtube.com/watch?v=Mz50NDtLs6Y (Merriam-Webster)
- **IPA**: /ˌhiː.moʊ.daɪˈæl.ə.sɪs/ (standard American)
- **Phonetic Spelling**: *hee-moh-dye-AL-uh-sis*

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3. Electrolyte

- **Pronunciation link**: https://www.merriam-webster.com/dictionary/electrolyte (How To Pronounce, Merriam-Webster)
- **IPA**: /ɪˈlek-trə-laɪt/ (Merriam-Webster)
- **Phonetic Spelling**: *ih-LEK-truh-lyt*

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4. Copper Sulfate

- **Pronunciation link**: https://dictionary.cambridge.org/us/pronunciation/english/copper-sulfate (Cambridge Dictionary)
- **IPA**: /ˌkɑː.pɚ ˈsʌl.feɪt/ (Cambridge Dictionary)
- **Phonetic Spelling**: *KAW-per SUL-fayt*

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5. Electromyography

- **Pronunciation link**: https://dictionary.cambridge.org/us/pronunciation/english/electromyography (Cambridge Dictionary)
- **IPA**: /ɪˌlek.troʊ.maɪˈɑː.grə.fi/ (American variant) (Cambridge Dictionary)

- **Phonetic Spelling**: *ih-LEK-troh-my-AG-ruh-fee*

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6. Electromyogram

- **Pronunciation link**: https://dictionary.cambridge.org/pronunciation/english/electromyogram (How To Pronounce, Cambridge Dictionary)
- **IPA**: /iˌlek.trəˈmaɪ.oʊ.græm/ (Cambridge Dictionary)
- **Phonetic Spelling**: *ih-lek-troh-MY-oh-gram*

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7. Autotroph

- **Pronunciation link**: ThoughtCo reference with breakdown (no link for pronunciation but included in list of difficult biology terms) (thoughtco.com)
- **IPA estimate**: /ˈɔː.tə.troʊf/
- **Phonetic Spelling**: *AW-tuh-trohf*

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8. Homeostasis

- **Pronunciation link**: Advanced Spelling & Vocabulary list (Merriam-Webster guided) (iew.com)
- **IPA**: /ˌhoʊ.mi.oʊˈsteɪ.sɪs/
- **Phonetic Spelling**: *hoh-mee-oh-STA-sis*