FINAL SCRIPT: APPROVED FOR FILMING

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Title: Thermal Preconditioning During Ex-vivo Lung Perfusion for the Rehabilitation of Damaged Lung Grafts before Transplantation

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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? **YES**

If **Yes**, can you record movies/images using your own microscope camera? **NO**

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**. **Our microscope has a camera port but we do not have a camera**

Carl Zeiss OPMI MDU 267067

2.2.1, 2.2.2, 2.3.1, 2.3.2, 2.4.1, 2.5.1, 2.5.2, 2.8.1, 2.11.1, 2.12.1, 2.12.2, 2.13.1, 2.14.1, 2.15.1, 2.15.2, 2.16.1, 2.18.1, 2.18.2, 2.19.1, 2.20.1, 2.20.2, 2.20.3.

Videographer: Please film all the SCOPE shots using the scope kit

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES**

Videographer: Please film all shots labeled SCREEN as a back up

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. **NO**

Current Protocol Length
Number of Steps: 32
Number of Shots: 54

Introduction

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

INTRODUCTION:

What is the scope of your research? What questions are you trying to answer?

1.1. **Anne Debonneville:** We work on ex-vivo lung perfusion to assess the possible therapeutic effects of transient heat exposure on damaged lung grafts.

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

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

1.2. **Anne Debonneville**: In the field of lung transplantation, ex-vivo lung perfusion and graft preservation at 10°C are the two major recent developments.

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

CONCLUSION:

What significant findings have you established in your field?

1.3. **Anne Debonneville:** Transient heat application during ex-vivo lung perfusion can recondition damaged lung grafts and make them suitable for subsequent transplantation.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.30.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Anne Debonneville:** In comparison to single-target pharmacological therapies, our protocol triggers an integrated physiological response targeting multiple pathophysiological insults.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: Figure 9*

What questions will future research focus on?

1.5. **Anne Debonneville:** Clinical translation of our findings will need to determine the optimal temperature and duration for heat application in human lungs.

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.

Ethics Title Card

This research has been approved by the local animal care committee

Protocol

NOTE: The author has reviewed all the footage and provided the timestamps. The author has reviewed the postshoot script as well. For screen captures, the postshoot note integrator (Swati) added the file name and timestamp and author reviewed them as well.

2. Detailed EVLP Protocol for Thermal Preconditioning

Demonstrator: Anne Debonneville

2.1. ~~Begin by opening the Basic Data Acquisition Software or BDAS *(B-Das)* and calibrating the pressure signals **[1]**. Calibrate the weight module by performing a two-point calibration **[2]**. Calibrate the pump speed by performing a 1-minute perfusate sampling at a flow rate of 10 milliliters per minute **[3]**.~~

2.1.1. ~~WIDE: Talent in front of the computer screen launching the application and calibrating the pressure signals~~

2.1.2. ~~Show the weight module calibration menu and perform a two-point calibration using 0 gram and 1 gram.~~ NOTE: These shots needs to be deleted and following additional steps-shots need to be added instead.

2.1.3. ~~Display the pump speed calibration interface and run a 1-minute sampling at 10 milliliters per minute.~~

2.2. To begin the surgical procedure, gently lift the skin of the anesthetized animal at the midline of the throat and make a 5-centimeter longitudinal incision along the caudal to cephalic axis **[1-TXT]**.

2.2.1. SCOPE: Talent lifting the throat skin at the midline. **TXT: Anesthesia: Induction with 5% isoflurane followed by ketamin/xylasin injection**

File name: SCOPE 00001

Timestamp: 00:10-00:17

NOTE: 2.2.2 is covered in 2.2.1 and narration has been edited.
Videographer: Please film the SCOPE shots using the scope kit

2.2.2. ~~SCOPE: Talent making a 5-centimeter incision from caudal to cephalic direction using small scissors.~~

2.3. Separate the salivary gland lobes **[1]** and, using curved forceps, carefully dissect the fascia over the sternohyoid muscles until the larynx and trachea are clearly exposed **[2]**.

2.3.1. SCOPE: Talent separating the salivary gland lobes.

File name : SCOPE 00001

Timestamp: 00:20-00:40

2.3.2. SCOPE: Talent using curved forceps to dissect the fascia and expose the larynx and trachea.

File name : SCOPE 00001

Timestamp:00:42-01:00

2.4. Then, pass a 4-0 silk suture underneath the trachea and prepare a pre-tied knot, keeping the forceps in place under the trachea **[1]**.

2.4.1. SCOPE: Talent passing a 4-0 silk suture under the trachea and forming a pre-tied knot with forceps still in position.

File name : SCOPE 00001

Timestamp: 01:00-01:29

2.5. Now, create a transverse incision between two cartilaginous rings of the trachea **[1]**. Insert a 2-millimeter outer diameter cannula into the incision and secure it by tightening the suture **[2]**.

2.5.1. SCOPE: Talent making a transverse incision between tracheal rings.

File name : SCOPE 00001

Timestamp: 01:37-01:41

2.5.2. SCOPE: Talent inserting the cannula into the tracheal incision and tightening the suture to secure the cannula in place.

File name : SCOPE 00001

Timestamp: 01:44-01: 48 + 02:00-02:29

2.6. Then, connect the cannula to the ventilator **[1]** and confirm effective ventilation by checking for the presence of bubbles in the water-filled container attached to the ventilator's expiratory arm **[2]**.

2.6.1. Talent connecting the tracheal cannula to the ventilator.

File name : B036C025 (2.6.1-1)

Timestamp: 00:13-00:18

2.6.2. Close-up of bubbles forming in the water-filled container connected to the expiratory arm of the ventilator.

File name : B036C026 (2.6.2-1)

Timestamp: 00:02-00:05

2.7. Using blunt-tipped scissors, incise the skin along the abdomen and enter the peritoneal cavity. Continue by incising the midline abdominal wall muscles up to the diaphragm **[1]**.

2.7.1. Talent using blunt-tipped scissors to incise the abdominal skin and enter the peritoneal cavity up to the diaphragm.

File name : B036C029 (2.7.1-1)

Timestamp: 00:04-00:46

2.8. Now, inject the prepared heparin solution into the vein and allow it to circulate for 5 minutes **[1-TXT]**.

2.8.1. SCOPE: Talent injecting heparin solution into a vein using a syringe. **TXT: Transect the vena cava and aorta to initiate exsanguination**

File name : SCOPE 00003

Timestamp: 00:23-00:28 + 00:35-00:38

2.9. Once the animal is fully exsanguinated and confirmed dead, stop the ventilation **[1]**. Disconnect the ventilator from the tracheal cannula to allow the lungs to deflate, marking the onset of warm ischemia **[2-TXT]**.

2.9.1. Talent turning off the ventilator.

File name : B036C031 (2.9.1-1)

Timestamp: 00:03-00:06

2.9.2. Talent detaching the ventilator tubing from the tracheal cannula. **TXT: Keep the total warm ischemic time to 1 h**

File name : B036C032 (2.9.2-1)

Timestamp: 00:04-00:10

2.10. After 45 minutes of warm ischemia, fill a 60-milliliter syringe with cold preservation solution **[1]**. Remove the bubbles along the tubes and immerse the tube in ice **[2]**.

2.10.1. Talent filling a 60 milliliter syringe with cold preservation solution.

File name : B036C033 (2.10.1-1)

Timestamp: 00:08-00:10 + 00:17-00:20

Added shot. Talent removing bubbles from the tubing and immersing it in ice.

File name : B036C034 (2.10.1-2)

Timestamp: 00:07-00:17 + 00:41-00:51 + 00:57-01:02 + 01:10-01:20

2.11. Using blunt-ended Lister scissors, perform a median sternotomy **[1]**. Carefully retract the chest wall using blunt retractors attached to elastic bands and a micro-mosquito hemostat secured to the diaphragm to expose the lungs **[2]**.

2.11.1. Talent performing a median sternotomy using blunt-ended Lister scissors.

File name : B036C036 (2.11.1-2.20.3-1)

Timestamp: 00:17-00:22 + 00:30-00:40

2.11.2. Talent retracting the chest wall using blunt retractors, elastic bands, and a micro-mosquito hemostat.

File name : B036C036 (2.11.1-2.20.3-1)

Timestamp: 01:02-01:32

2.12. Next, make an "X" shaped incision at the apex of the left ventricle **[1]**. Slide the scissors inside the heart and extend the incision up to the left atrium to allow for later insertion of the left atrial cannula **[2]**.

2.12.1. SCOPE: Talent making an "X" incision at the apex of the left ventricle.

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 03:25-03:34

2.12.2. SCOPE: Talent inserting scissors into the left ventricle and extending the incision toward the left atrium.

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 03:49-04:00

2.13. Then, pass a 5-0 silk suture underneath the main pulmonary artery, prepare a pre-tied double knot, and hold one end of the suture using a home-made holder **[1]**.

2.13.1. SCOPE: Talent passing a 5-0 silk suture under the main pulmonary artery and forming a double knot while holding one end of the suture

File name and Timestamp: SCOPE 00004 (2.11.1-2.23) 04:09-04:24 + 04:36-

04:40 + B036C036 (2.11.1-2.20.3-1) 04:48-04:53

2.14. Prepare an additional suture around the heart to be used later for securing the left atrial cannula **[1]**.

2.14.1. SCOPE: Talent placing a suture around the heart and positioning it for later cannula securement.

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 05:19-05:38

2.15. Next, make a small incision in the main pulmonary artery **[1]**. Insert the pulmonary artery cannula carefully, ensuring that no air bubble enters the system **[2]**. ~~Immediately perfuse the lungs with 25 milliliters of cold preservation solution through the pulmonary artery cannula to initiate cold ischemia time **[3]**.~~

2.15.1. SCOPE: Talent making a small incision in the main pulmonary artery.

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 06:19-06:26

2.15.2. SCOPE: Talent inserting the pulmonary artery cannula while avoiding air bubble introduction. **TXT: Immediately perfuse the lungs with 25 mL cold preservation solution via the pulmonary artery to begin cold ischemia**

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 06:44-06:52

~~2.15.3. Talent perfusing the lungs with 25 milliliters of cold preservation solution.~~

2.16. Secure the pulmonary artery cannula in place using the pre-tied suture **[1]**.

2.16.1. SCOPE: Talent tightening the pre-tied suture to fix the pulmonary artery cannula.

File name : SCOPE 00004 (2.11.1-2.23 not written on video, but we hear it)

Timestamp: 07:21-07:39

2.17. Immediately after pulmonary artery cannulation, reduce the respiratory rate on the ventilator to 25 breaths per minute **[1]** and reconnect the tracheal cannula to the ventilator **[2]**.

2.17.2 Show ventilator control panel as the respiratory rate is adjusted to 25 breaths per minute.

File name : B036C035 (2.17.2-1)

Timestamp: 00:29-00:35 + 00:44 Author's NOTE: We should see the rate at 25.

2.17.1 Talent reconnecting the tracheal cannula to the ventilator tubing. NOTE: 2.17.1 and 2.17.2 are inverted along with VO

File name : B036C036 (2.11.1-2.20.3-1)

Timestamp: 08:17-08:21

2.18. Insert the left atrial cannula through the incision made in the left ventricle and advance it into the left atrium **[1]**. Secure the cannula using the second pre-tied suture placed around the heart **[2]**.

2.18.1. SCOPE: Talent inserting the cannula through the ventricular incision and positioning it in the left atrium.

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 08:48-08:57

2.18.2. SCOPE: Talent tightening the second suture to secure the left atrial cannula**.**

File name : SCOPE 00004 (2.11.1-2.23)

Timestamp: 09:25-09:30 + 09:35-09:37 + 09:40-10:09

2.19. At the end of the perfusion with the preservation solution, clamp the pulmonary artery cannula using a bulldog clamp and disconnect the cannula from the tubing **[1]**.

2.19.1. SCOPE: Talent applying a bulldog clamp to the pulmonary artery cannula and disconnecting it.

File name : B036C038 (2.19.1-2.20.3-2)

Timestamp: 00:17-00:24

2.20. While the lungs are fully inflated, clamp the trachea using an aneurysm clip **[1]**. Dissect the descending aorta and other supporting vessels to free the heart-lung block**,** lifting it gently by grasping the trachea **[2]**. Place the heart-lung block in a dish filled with preservation solution in a prone position **[3-TXT]**.

2.20.1. SCOPE: Talent applying an aneurysm clip to the trachea while lungs are fully inflated.

File name : SCOPE 00006 (2.19.1-2.23 take 2)

Timestamp: 00:34-00:41

2.20.2. Talent dissecting the descending aorta and supporting vessels with precision. Author's NOTE: The scope was out of the field for this step, so I had to use an alternative view. It is not optimal, but I believe the essential details are still visible

File name : B036C038 (2.19.1-2.20.3-2)

Timestamp: 02:40-03:00

2.20.3. ~~SCOPE: Talent gently lifting the heart-lung block by holding the trachea~~. Author's NOTE: This step can be removed as it is part of the previous step and is the description of how to do it.

2.20.4. Talent placing the heart-lung block into a preservation solution-filled dish in the correct position. **TXT: Store at 4 °C for a total cold ischemia time of 1 h**

File name : B036C039 (2.20.4-1)

Timestamp: 00:09-00:15 + 00:27-00:41

2.21. Place an ice bucket inside a thermostatic water bath to reduce the perfusate temperature to 15 degrees Celsius **[1]**.

2.21.1. Talent placing an ice bucket into the thermostatic water bath.

File name : B036C040 (2.21.1-1)

Timestamp: 00:05-00:10

2.22. Now, fill the reservoir with 100 milliliters of perfusate and allow it to circulate through the system **[1]**.

2.22.1. Talent pouring 100 milliliters of perfusate into the reservoir.

File name : B036C041 (2.22.1-1)

Timestamp: 00:25-00:37

2.23. Before connecting the heart-lung block on the E-V-L-P, fill the bubble trap **[1]** then start by connecting the ventilation cannula while keeping the aneurysm clip in place **[2]**, then connect the pulmonary artery cannula, ensuring no air bubbles enter the lung vasculature **[3]**. Allow a few drops of perfusion solution to exit from the left atrial cannula and connect it **[4]**.

Added shot: Talent filling the bubble trap.

File name : B036C043 (2.23.1-1)

Timestamp: 00:06-00:16

Added shot: Talent connecting the ventilation cannula while keeping the aneurysm clip in place.

File name : B036C044 (2.23.2-1)

Timestamp: 00:21-00:26

2.23.1. Talent connecting the pulmonary artery cannula carefully ensuring no air bubbles enter the lung vasculature.

File name : B036C044 (2.23.2-1)

Timestamp: 00:29-00:49

2.23.2. Talent connecting the left atrial cannula after confirming solution flow.

File name : B036C044 (2.23.2-1)

Timestamp: 01:05-01:15

2.24. Zero the weight module and monitor the change in weight of the heart-lung block throughout the ex vivo lung perfusion **[1]**. Start perfusion at 2% of the calculated theoretical cardiac output **[2-TXT]**.

2.24.1. WIDE: Talent in front of the transducer/amplifier as the weight module is zeroed.

File name and timestamp : B036C045 (2.24.1-1) 00:05-00:12 + B036C046 (2.23.2-1) 00:00-00:02

2.24.2. Talent initiating perfusion at 2% of the calculated cardiac output. **TXT: Gradually increase the perfusate flow and temperature**

File name : B036C047 (2.24.2-1)

Timestamp: 00:08-00:13

2.25. Adjust the temperature of the perfusate by progressively increasing the temperature of the water bath **[1]**.

2.25.1. Talent adjusting the thermostat on the water bath to raise the temperature.

File name : B036C048 (2.25.1-1)

Timestamp: 00:05-00:16

2.26. Once the perfusate temperature reaches 35 degrees Celsius, remove the aneurysm clip from the trachea **[1]**. Start the ventilator and after 10 minutes at this setting, adjust ventilation to 15 strokes per minute and 6 milliliters per kilogram **[3-TXT].**

2.26.1. Talent removing the aneurysm clip from the trachea.

File name : B036C049 (2.26.1-1)

Timestamp: 00:19-00:28

2.26.2. Talent starting ventilation for ex vivo lung ventilation

File name : B036C050 (2.26.2-1)

Timestamp: 00:15-00:30

and SCREEN: JoVE_SCREEN_69084_step-2.26.2.-Start-ventilation.mp4: 00:02 – 00:05.

Video editor: IF possible, show both shots side by side on 1 screen. NOTE: Shot action has been edited.

Added shot: SCREEN: JoVE_SCREEN_69084_step 2.26.3 Set to 15 stroke and 6 ml: 00:00 – 00:12 **TXT: Gradually increase the rate and tidal volume**

2.27. Set the ventilator dedicated for ex vivo ventilation at a respiratory rate and tidal volume to 7 strokes per minute, 3 milliliters per kilogram and the positive end-expiratory pressure at 3 centimeters of water [1]. **Author's NOTE:** Move shot 2.27 above 2.26 as the ventilator must be set before starting the ventilation.

2.27.1. SCREEN: 2.27.1_A-set-ventilation.mp4 :02 – 00:07, 00:18 – 00:21. *Video editor: Between 00:02 – 00:07, emphasize "7/min" and "3 mL/kg" when VO mentions the respective values.*

Videographer: Please film all shots labeled SCREEN as a back up

2.28. Every 30 minutes during ex vivo lung perfusion, apply a recruitment maneuver by setting the airway pressure at 15 centimeters of water for 6 seconds to prevent atelectasis **[1]**.

2.28.1. SCREEN: 2.28.1-recruitment-maneuver.mp4: 00:01 – 00:14 and B036C053 (2.28.1 & 2.29.1-3): 00:30-00:41 **NOTE:** Show both videos side by side.

2.29. At 1 hour of ex vivo lung perfusion, perform a stepwise lung inflation up to a total lung volume of 10 milliliters per kilogram of body weight to measure lung compliance and peak inspiratory pressure **[1]**.

2.29.1. SCREEN: 2.29.1-compliance.mp4: 00:00 – 00:30 and B036C053: 01:40-02:00 **NOTE:** Show both videos side by side.

2.30. After 60 minutes, initiate the ex vivo heating protocol. Increase the temperature of the water bath up to 43 degrees Celsius to achieve 41.5 degrees Celsius at the inflow pulmonary artery cannula **[1]**.

2.30.1. Show temperature display on the water bath increasing up to 43 degrees Celsius and inflow cannula temperature reaching 41.5 degrees Celsius.

File name : B036C054 (2.30.1-1)

Timestamp: 00:05-00:14 and 02:52-02:55

2.31. After 30 minutes of heating, add ice to the water bath to quickly reduce the temperature at the inflow cannula to 37 degrees Celsius within 5 minutes **[1-TXT]**. Collect samples from one of the sampling ports connected to the circuit of the perfusate solution at 1 hour and 2 hours, 3 hours, 4.5 hours and at 6 hours during the perfusion period **[2]**.

2.31.1. Talent adding ice to the water bath and screen showing temperature drop to 37 degrees Celsius at the inflow cannula. **TXT: Keep perfusate temperature at 37 °C until the end of the EVLP**

File name : B036C055 (2.31.1-1)

Timestamp: 00:04-00:22 and 03:21-03:37

2.31.2. Talent drawing perfusate samples into labeled tubes at designated time points.

File name : B036C056 (2.31.2-1)

Timestamp: 00:15-00:20 and 02:23-02:32

2.32. After 6 hours of ex vivo lung perfusion, reapply the aneurysm clip to the trachea **[1]**. Clamp the pulmonary artery cannula with a bulldog clamp **[2]** and carefully dissect the lungs by separating the individual lobes **[3-TXT]**.

2.32.1. Talent applying the aneurysm clip to the trachea with the lung fully expanded.

File name : B036C057 (2.32.1-1)

Timestamp: 00:47-00:54

2.32.2. Talent clamping the pulmonary artery cannula with a bulldog clamp.

File name : B036C059 (2.32.2-2)

Timestamp: 00:05-00:10

2.32.3. Talent gently separating the lung lobes using dissection tools. **TXT: Snap-freeze in Liq. N_2 and store at –80 °C or fix in PFA**

File name : B036C060 (2.32.3-1)

Timestamp: 00:50-01:03 + 01:25-01:30 + 01:52-02:01 + 02:04-02:15 + 02:18-02:29

Results

3. Results

3.1. The static pulmonary compliance ratio was significantly improved in the thermal preconditioning group compared to the control at 4.5 hours **[1]** and 6 hours of ex vivo lung perfusion **[2].**

3.1.1. LAB MEDIA: Figure 9A. *Video editor: Highlight the higher open circle data point at 4.5 hours on the y-axis labeled "SPC ratio (end EVLP/1h)"*

3.1.2. LAB MEDIA: Figure 9A. *Video editor: Highlight the higher open circle data point at 6 hours on the y-axis labeled "SPC ratio (end EVLP/1h)"*

3.2. Lung weight gain during ex vivo lung perfusion was significantly lower in the thermal preconditioning group compared to control at 6 hours **[1].**

3.2.1. LAB MEDIA: Figure 9B. *Video editor: Highlight the lower open circle at the 6 hour timepoint.*

3.3. Thermal preconditioning significantly reduced von Willebrand factor levels in perfusate at the end of ex vivo lung perfusion compared to control **[1].**

3.3.1. LAB MEDIA: Figure 9C. *Video editor: Highlight the lower open circle at the 4.5-hour timepoint "*

3.3.2. LAB MEDIA: Figure 9C. *Video editor: Highlight the lower open circle at the 6-hour timepoint "*

3.4. Lung histology showed reduced structural damage in thermal preconditioning group **[1],** as confirmed by significantly lower lung injury scores **[2].**

3.4.1. LAB MEDIA: Figure 9D.

3.4.2. LAB MEDIA: Figure 9E. *Video editor: Highlight the shorter bar for TP in the graph labeled "Lung histology score"*

3.5. The expression of HSP70 in the thermal preconditioning group peaked after 3 hours of ex vivo lung perfusion **[1]** and remained stably elevated at 4.5 hours **[2]** and 6 hours **[3].**

3.5.1. LAB MEDIA: Figure 9F. *Video editor: Highlight the peak open circle data point at 3 hours*

3.5.2. LAB MEDIA: Figure 9F. *Video editor: Highlight the open circle data point at 4.5 hours*

3.5.3. LAB MEDIA: Figure 9F. *Video editor: Highlight the open circle data point at 6 hours, showing continued elevation in the TP group*