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Title: Bidirectional Electrical and Optoelectronic Interfaces in Healthy and Ischemic *Ex Vivo* Rat Hearts

Authors and Affiliations:
Pengju Li[1]*, Jing Zhang[2]*, Chuanwang Yang[2]*, Zhe Cheng[3], Junyi Yin[1], Bozhi Tian[2,3,4]

[1]Pritzker School of Molecular Engineering, The University of Chicago
[2]The James Franck Institute, The University of Chicago
[3]Department of Chemistry, The University of Chicago
[4]The Institute for Biophysical Dynamics, The University of Chicago

***These authors contributed equally to this work**

Corresponding Authors:
Pengju Li (pengju@uchicago.edu)
Bozhi Tian (btian@uchicago.edu)
Email Addresses for All Authors:
Pengju Li (pengju@uchicago.edu)
Jing Zhang (jingzhang1209@uchicago.edu)
Chuanwang Yang (chuanwangyang@uchicago.edu)
Zhe Cheng (zhecheng@uchicago.edu)
Junyi Yin (junyiyin@uchicago.edu)
Bozhi Tian (btian@uchicago.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? **Yes, all done**

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

Current Protocol Length
Number of Steps: 21
Number of Shots: 49

Introduction

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

1.1. **Pengju Li:** We developed an *ex vivo* heart model to evaluate optoelectronic and electronic materials for cardiac stimulation and sensing, bridging benchtop innovation with translational bioelectronic applications.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.3*

What research gap are you addressing with your protocol?

1.2. **Pengju Li:** We bridge the gap between *in vitro* and *in vivo* by offering a controlled, nongenetic *ex vivo* heart model to evaluate both wired and wireless bioelectronic materials in real tissue.

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

What advantage does your protocol offer compared to other techniques?

1.3. **Zhe Cheng:** Our protocol enables fast, reproducible testing of materials in whole beating hearts, combining physiological relevance with precise control—ideal for comparing stimulation and sensing performance across different devices.

1.3.1. 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?

1.4. **Chuanwang Yang:** We will explore therapeutic materials that modulate heart function and validate their efficacy in treating myocardial infarction using our ischemia-reperfusion ex vivo model.

1.4.1. 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 ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Pengju Li, Dr.**: Publishing with JoVE enhances visibility through visual, accessible science. It supports reproducibility, enabling labs worldwide to adopt our protocol, even with limited resources. This broadens the impact of our work, advancing education, collaboration, and innovation in cardiac bioelectronics.

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

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Chicago

Protocol

2. Heart Extraction and Preparation for Langendorff Perfusion

Demonstrator: Pengju Li

2.1. To begin, confirm general anesthesia by pinching one of the paws of the anesthetized rat and proceed only if no response is observed **[1]**.

2.1.1. WIDE: Talent pinching the rat's paw and pausing to observe for any response.

2.2. Using dissection T-pins, secure the upper and lower arms of the rat to the surgical board **[1]**. Then make a 5-centimeter incision just below the chest to open the ribcage **[2]**. Using scissors, carefully cut through the diaphragm to expose the heart and lungs **[3]**.

2.2.1. Talent placing and securing the arms of the rat to the board with dissection T-pins.

2.2.2. Talent making a long incision below the chest using scissors.

2.2.3. Talent cutting through the diaphragm to expose thoracic organs.

2.3. Now cut through the ribs on both sides to fully open the ribcage **[1]**. Secure the opened ribcage at the sternum using a hemostat **[2]**. Use a second hemostat to hold the vena cava from below the heart **[3]**.

2.3.1. Talent cutting through the ribs on both sides with scissors.

2.3.2. Talent pinning back the ribcage at the sternum using a hemostat.

2.3.3. Talent placing a second hemostat under the heart to hold the vena cava.

2.4. Cut under the hemostat with bent blunt scissors as close to the bottom of the rib cage as possible, to remove the heart **[1]**. Transfer the heart immediately into a Petri dish filled with ice-cold HBSS *(H-B-S-S)* **[2]**.

2.4.1. Talent removing the heart with bent blunt scissors.

2.4.2. Talent placing the heart into the HBSS-filled Petri dish.

2.5. Fill a cannula and a separate Petri dish completely with ice-cold HBSS **[1-TXT]**. Transfer the heart to the Petri dish prefilled with the buffer for sectioning **[2]**.

2.5.1. Talent carefully filling a cannula and Petri dish with cold HBSS. **TXT: Ensure that no bubbles are present in the Langendorff perfusion system**

2.5.2. Talent transferring the heart to the HBSS-filled Petri dish.

2.6. Use tweezers or scissors to remove the lungs and other connective tissues attached to the heart **[1]**.

2.6.1. Talent using tweezers and/or scissors to remove lungs and connective tissues.

2.7. Then press on the heart gently and trace the blood path to locate the aorta **[1]**. If the aortic arch is preserved, cut under the first ascending artery **[2]**. Cannulate the aorta carefully **[3]**.

2.7.1. Shot of the heart being pressed and blood flow being seen.

2.7.2. Talent making an incision below the first ascending artery.

2.7.3. Talent inserting the cannula into the aorta and securing it with surgical sutures.

3. Langendorff Perfusion Setup and Initial Monitoring

Demonstrator: Pengju Li, Zhe Cheng

3.1. Prime the Langendorff apparatus with pre-oxygenated working solution **[1]**. Open the buffer flow **[2]** and attach the cannula to the perfusion apparatus carefully, ensuring that no air bubbles enter the system **[3]**. Observe the heart begins to contract once perfusion starts **[4]**.

3.1.1. Talent filling the Langendorff system with pre-oxygenated buffer.

3.1.2. Shot of the buffer flow being opened.

3.1.3. Talent connecting the cannula to the apparatus while buffer flows.

3.1.4. Shot of the heart starting to contract.

3.2. Now cut off part of the atria or atrial appendage using small scissors **[1]**. Before inserting the balloon into the left ventricle, deflate it completely **[2]**. Then use the connected syringe to fill the balloon with water **[3]**.

3.2.1. Talent removing part of the atria (or atrial appendage) from the heart.

3.2.2. Talent deflating the balloon.

3.2.3. Talent inserting the balloon into the left ventricle and filling it with water.

3.3. Next, connect BP-100 *(B-P-One-Hundred)* pressure probes to the perfusion line and the water-filled balloons to monitor left ventricular pressure, respectively **[1]**.

3.3.1. Talent attaching BP-100 probes and balloon to monitor LVP.

3.4. Amplify all signal outputs including left ventricular pressure and ECG using the IA-400D *(I-A-Four-Hundred-D)* amplifier **[1]**.

3.4.1. Talent turning on the IA-400D amplifier.

3.5. Monitor the pressure of the HEPES *(He-Pees)* Tyrode's buffer and adjust it to stay within the optimal range of 80 to 100 millimeters of mercury **[1]**. Modify the volume of water in the balloon using the syringe **[2]** to set the baseline left ventricular pressure to approximately 20 millimeters of mercury **[3]**.

3.5.1. Talent observing the buffer pressure gauge and adjusting the regulator.

3.5.2. Talent injecting or withdrawing water from the balloon to calibrate the LVP baseline.

3.5.3. SCREEN: 68305_screenshot_1.mp4 00:00-00:16

3.6. Connect the electrocardiogram electrodes by grounding the cannula **[1]** and placing the electrode wires at the sides, top, or apex of the heart based on user preference **[2]**.

3.6.1. Talent grounding the cannula.

3.6.2. Talent positioning the ECG electrodes on different areas of the heart.

AND
SCREEN: 68305_screenshot_2.mp4. 00:00-00:19
Video Editor: Please play both shots side by side

4. Myocardial Infarction Induction, Reperfusion Monitoring, and Bidirectional Interface Modulation and Recording

Demonstrator: Pengju Li, Chuanwang Yang

4.1. Fill the heated chamber with HEPES Tyrode's buffer pre-warmed to 37 degrees Celsius **[1]**. Submerge the heart into the chamber and turn the stopcock to halt buffer flow, inducing global ischemia **[2-TXT]**.

4.1.1. Talent pouring 37 degrees Celsius buffer into the heated chamber.

4.1.2. Talent placing the heart into the buffer chamber and adjusting the stopcock to stop flow. **TXT: Adjust the ischemia duration based on experimental requirements**

4.2. After 30 minutes of ischemia, turn the stopcock to restore buffer flow and initiate

reperfusion **[1]**. Allow the heart to perfuse for 45 minutes before terminating the experiment for infarction staining **[2]**. Verify the success of ischemia by observing a reduction in heart rate and irregular ECG signals **[3]**.

4.2.1. Talent removing heart from the buffer chamber and turning the stopcock to restart the buffer flow.

4.2.2. Shot of the heart as it continues perfusion during reperfusion period.

4.2.3. SCREEN: 68305_screenshot_3.mp4 . 00:00-00:17

4.3. To establish bidirectional optoelectrical interfaces between devices and heart tissue place a silicon optoelectronic membrane onto the desired stimulation site on the heart **[1]**. Allow it to self-attach to the epicardium using capillary force **[2]**.

4.3.1. Talent aligning and placing the silicon membrane on the heart.

4.3.2. Close-up of the membrane attached to the epicardium.

4.4. Connect the electrodes to the RHD *(R-H-D)* recording system or a compatible electrophysiological platform **[1]**. Then position the flexible multielectrode arrays on the ventricular surfaces of the isolated heart **[2]**.

4.4.1. Talent connecting the electrode leads to the RHD interface.

4.4.2. Talent placing multielectrode arrays spanning the left and right ventricular surfaces.

4.5. Now program the 635-nanometer laser source with the desired frequency and duty cycle, using transistor-transistor logic signals **[1-TXT]**. Focus the laser beam to a 1-millimeter spot and align it over the silicon membrane **[2]**.

4.5.1. SCREEN: 68305_screenshot_4.mp4. 00:00-00:24
TXT: Set frequency to 4 Hz with 10 ms pulse duration, indicating 4% duty cycle.

4.5.2. Talent adjusting laser focus to target the membrane with a 1 mm spot.

4.6. Start the recording and stimulation protocol **[1]**. Gradually increase the laser intensity **[2]** until continuous override pacing of the heart is observed **[3]**.

4.6.1. Shot of the laser stimulation program started.

4.6.2. Talent increasing the laser intensity by adjusting the dial.

4.6.3. Shot of the heart being paced at 4 Hz frequency.

AND
SCREEN: 68305_screenshot_5_1.mp4. 00:00-00:10

SCREEN: 68305_screenshot_5_2.mp4. 00:00-00:10

Video Editor: Please show all shots side by side if possible

4.7. To establish bidirectional electrode-based electrical interfaces between devices and heart tissue, connect the stimulation electrodes in a two-electrode configuration, placing a porous carbon working electrode on the left ventricular wall **[1]** and a counter electrode on the right ventricular wall **[2]**.

4.7.1. Talent placing the working electrode on the left ventricular wall.

4.7.2. Shot of the counter electrode being placed on the right ventricular walls, respectively.

4.8. Using a potentiostat, deliver square current waveforms such as 2 milliampere with 1 millisecond pulse duration until successful pacing of the heart is achieved **[1]**.

4.8.1. SCREEN: 68305_screenshot_6.mp4 00:00-00:06
AND

Shot of the heart being paced to beat at 4 Hz frequency.

Video Editor: Please show both shots side by side

Results

5. Results

5.1. A nanoporous carbon-based platform for effective electrical modulation or sensing of cardiac systems was established **[1]**.

5.1.1. LAB MEDIA: Figure 10A. Video editor: Show the image of the flexible electrode device being positioned on the heart.

5.2. Upon 4 hertz stimulation at 1 milliampere, both gold and nanoporous carbon-coated gold electrodes achieved effective overdrive pacing **[1]**, with higher electrocardiogram amplitudes observed in the nanoporous carbon group **[2]**.

5.2.1. LAB MEDIA: Figure 10B. *Video editor: Highlight the red trace (Au) and the blue trace (Au-C)*

5.2.2. LAB MEDIA: Figure 10B. *Video editor: Highlight the Au-C waveline*

5.3. Both electrodes demonstrated an exponential decrease in threshold current with increasing pulse duration **[1]**.

5.3.1. LAB MEDIA: Figure 10C. *Video editor: Highlight the two curves labeled Au and Au-C*

5.4. At a stimulation current of 4 milliamperes per square centimeter and 1 millisecond duration, the threshold voltage required for effective pacing was 1.32 volts for gold electrodes **[1]** and 0.90 volts for nanoporous carbon-coated electrodes **[2]**.

5.4.1. LAB MEDIA: Figure 10D. *Video editor: Highlight the red curve between 1.0 and 1.5*

5.4.2. LAB MEDIA: Figure 10D. *Video editor: Highlight the blue curve between 0.5 and 1.0*

5.5. A 16-channel nanoporous carbon-grafted gold electrode significantly enhanced epicardial electrocardiogram signal detection **[1]**, improving the signal-to-noise ratio by 8-fold compared to standard gold electrodes **[2]**.

5.5.1. LAB MEDIA: Figure 10E. *Video editor: Highlight the Au-C blue trace*

5.5.2. LAB MEDIA: Figure 10F. *Video editor: Emphasize the boxplot labeled "Au-C"*

5.6. Successful induction of ischemia-reperfusion infarction was validated post-experimentally by TTC staining **[1]**, where infarcted hearts displayed distinct white regions representing myocardial damage **[2]**.

5.6.1. LAB MEDIA: Figure 7

5.6.2. LAB MEDIA: Figure 7B. *Video editor: Highlight the white areas*

5.7. During real-time monitoring, ischemic hearts exhibited significantly reduced heart rate **[1]**.

5.7.1. LAB MEDIA: Figure 7A. *Video editor: Highlight the LVP and ECG waveforms under the "30 min ischemia and 50 min reperfusion" column*

5.8. Multielectrode array mapping of ischemic hearts revealed decreased electrical conduction velocity across the epicardium, indicated by increased signal delay **[1]**.

5.8.1. LAB MEDIA: Figure 7A. *Video editor: Highlight the bottom right conduction map showing extended red zones*

Pronunciation Guide:

- **Langendorff**
  - Pronunciation link: No confirmed link found
  - IPA: /ˈlɑːŋənˌdɔːrf/
  - Phonetic Spelling: LAHNG-uhn-dorf
- **HEPES**
  - Pronunciation link: No confirmed link found
  - IPA: /ˈhiːpiːz/
  - Phonetic Spelling: HEE-peez
- **Tyrode's**
  - Pronunciation link: https://www.merriam-webster.com/medical/Tyrode%20solution
  - IPA: /ˈtaɪˌroʊdz/
  - Phonetic Spelling: TIE-rohdsmerriam-webster.com+12merriam-webster.com+12merriam-webster.com+12
- **Potentiostat**
  - Pronunciation link: No confirmed link found
  - IPA: /pəˈtɛnʃiəˌstæt/
  - Phonetic Spelling: puh-TEN-shee-uh-stat
- **Epicardium**
  - Pronunciation link: https://www.merriam-webster.com/dictionary/epicardium
  - IPA: /ˌɛpɪˈkɑːrdiəm/
  - Phonetic Spelling: eh-pih-KAR-dee-ummerriam-webster.com+3merriam-webster.com+3merriam-webster.com+3
- **Ischemia**
  - Pronunciation link: https://www.merriam-webster.com/dictionary/ischemia
  - IPA: /ɪˈskiːmiə/
  - Phonetic Spelling: ih-SKEE-mee-uhmerriam-webster.com+5merriam-webster.com+5merriam-webster.com+5
- **Reperfusion**
  - Pronunciation link: https://www.merriam-webster.com/dictionary/reperfusion
  - IPA: /ˌriːpərˈfjuːʒən/
  - Phonetic Spelling: ree-per-FYOO-zhun
- **Nanoporous**
  - Pronunciation link: No confirmed link found
  - IPA: /ˌnænoʊˈpɔːrəs/
  - Phonetic Spelling: NAN-oh-por-us
- **Electrophysiological**
  - Pronunciation link: No confirmed link found
  - IPA: /ɪˌlɛktroʊˌfɪziəˈlɑːdʒɪkəl/
  - Phonetic Spelling: ih-LEK-troh-FIZ-ee-uh-LOJ-ih-kul
- **Transistor-Transistor Logic**

- Pronunciation link: No confirmed link found
- IPA: /trænˈzɪstər trænˈzɪstər ˈlɒdʒɪk/
- Phonetic Spelling: tran-ZIS-ter tran-ZIS-ter LOJ-ik