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Title: High-Throughput Capable Three-Dimensional Tissue Model for Quantification of Electroporation Thresholds

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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

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? **Yes, all done**

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: **07/21/2025**

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

Current Protocol Length

Number of Steps: 25
Number of Shots: 45 (3 SC)

Introduction

1.1. **Robert Williamson:** Our research explores clinical uses for electroporation using microsecond-duration, bipolar waveforms. We have previously investigated these waveforms for soft tissue ablation and are now optimizing for in vivo transfection.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What advantage does your protocol offer compared to other techniques?

1.2. **Alexia Cash:** This protocol enables more efficient reversible and irreversible electroporation threshold identification than cuvette-based approaches and in more in-vivo-representative conditions than other 2D and 3D in vitro models.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.2.1*

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

1.3. **Robert Williamson:** The thresholds identified using this model inform our parameter choices in vivo to determine how best to deliver things such as DNA vaccines, CRISPR components, and other macromolecules via electroporation.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 6.2.1*

Protocol

NOTE: LAB MEDIA/SCREEN/SCOPE timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sulakshana Karkala) for queries regarding lab media.

2. 3D Tissue Model Creation

Demonstrator: Robert Williamson

2.1. To begin, place the cell suspension and the required reagents in a biosafety cabinet **[1]**. Thoroughly mix the prepared cell suspension with Type I Bovine Collagen Solution in a 1 to 1 ratio **[2-TXT]**. Place the mixture on ice or into a cold bead bath to prevent premature polymerization **[3]**.

2.1.1. WIDE: Talent placing the cell suspension and reagent tubes inside a biosafety cabinet.

2.1.2. Talent mixing cell suspension with collagen solution in a 1:1 ratio by pipetting. **TXT: Cell suspension: 8 × 10^6 cells/mL; Bovine Collagen Solution: 3 mg/mL**

2.1.3. Talent placing the mixed solution on ice.

2.2. Then, pipette 500 microliters of the combined solution to coat the bottom of each well of a 12-well plate **[1]**. Gently swirl the plate to ensure that the gel contacts the walls of each well **[2]**.

2.2.1. Talent pipetting 500 microliters of the cell-collagen mixture into each well of a 12-well plate.

2.2.2. Talent gently swirling the 12-well plate to distribute the gel.

2.3. Incubate the gels in a humidified incubator set at 37 degrees Celsius with 5 percent carbon dioxide for 6 hours or until the gels become firm **[1]**.

2.3.1. Talent placing the plate in a humidified incubator set to 37 degrees Celsius and 5 percent carbon dioxide.

2.4. Next, tilt the well plate and gently add 500 microliters of culture medium to each well, letting it slide down the wall of the plate **[1-TXT]**.

2.4.1. Talent tilting the plate and slowly adding 500 microliters of culture medium down the wall of each well. **TXT: Perform at least 1 h before treatment**

3. Electrode Fabrication Before Treatment

Demonstrator: Alexia Cash

3.1. Remove the plastic Luer connection from two 1.64-millimeter 304 stainless steel blunt-tip syringe needles **[1]**. Set one needle aside to serve as the pin electrode **[2]**. For the other needle, flatten the last 5 millimeters of one end **[3]**.

3.1.1. Talent removing the plastic Luer connections from two stainless steel needles.

3.1.2. Talent setting aside one of the needles as the pin electrode.

3.1.3. Talent using a tool to flatten the last 5 millimeters of the other needle.

3.2. Then, cut a section of 19-millimeter outer diameter 316 stainless steel tubing long enough to sit flush against the bottom of a well plate to create the ring electrode **[1]**.

3.2.1. Talent cutting a section of 19-millimeter tubing to the correct length to serve as a ring electrode.

3.3. Design an electrode holder using CAD software to fit the electrode components **[1]**.

3.3.1. LAB MEDIA: Figure 1.

3.4. Fit the ring and pin electrodes into the electrode holder to assemble the electrode **[1]** and press-fit the needle with the flattened end into the holder to secure the ring electrode **[2]**.

3.4.1. Talent inserting the ring and pin electrodes into the fabricated holder.

3.4.2. Talent securing the ring electrode by press-fitting the flattened needle into the electrode holder.

4. Treatment of 3D Tissue Model with Electroporation

Demonstrator: Robert Williamson

4.1. In a biosafety cabinet, tilt the prepared plate **[1]** and aspirate 400 microliters of culture medium from each well **[2]**. Add 20 microliters of 5 micrograms per microliter green fluorescent protein plasmid solution to the aspirated wells **[3]**.

4.1.1. Talent tilting the 12-well plate inside a biosafety cabinet.

4.1.2. Talent aspirating 400 microliters of culture medium from each well.

4.1.3. Talent adding 20 microliters of green fluorescent protein plasmid solution into the aspirated wells.

4.2. Gently swirl the plate to ensure the solution spreads evenly across the gel surface **[1]**.

4.2.1. Talent gently swirling the plate.

4.3. Incubate the gels in a humidified incubator set at 37 degrees Celsius with 5 percent carbon dioxide for 10 minutes **[1]**.

4.3.1. Talent placing the plate into a humidified incubator set to 37 degrees Celsius with 5 percent carbon dioxide.

4.4. Next, insert the fiber-optic temperature probe into the pin electrode and begin recording the temperature **[1]**.

4.4.1. Talent inserting the fiber-optic temperature probe into the pin electrode

4.5. Connect the positive lead of the electroporator to the pin electrode **[1]** and the negative lead to the needle securing the ring electrode **[2]**.

4.5.1. Talent attaching the positive lead of the electroporator to the pin electrode.

4.5.2. Talent connecting the negative lead to the needle that secures the ring electrode.

4.6. Now, turn on the hot plate and heat the gels to maintain a temperature of 37 degrees Celsius **[1]**.

4.6.1. Talent switching on the hot plate and adjusting the setting to keep the gel temperature at 37 degrees Celsius.

4.7. Then, insert the assembled ring-and-pin electrode with the temperature probe into the well **[1]** and ensure the gel has reached a temperature of 37 degrees Celsius **[2]**.

4.7.1. Talent carefully lowering the electrode assembly with the probe into a well.

4.7.2. Display the fiber-optic temperature probe reading showing a stable temperature of 37 degrees Celsius.

4.8. Activate the electroporator to deliver the treatment **[1]**. Then, add 100 microliters of

culture medium to any gels that appear dry and repeat the electroporation treatment steps **[2]**.

4.8.1. Talent activating the electroporator to deliver electroporation pulses to the gel.

4.8.2. Talent inspecting the wells and adding 100 microliters of medium to the ones that show signs of dryness.

4.9. Once all treatments are completed, incubate the gels in a humidified incubator at 37 degrees Celsius with 5 percent carbon dioxide for 10 minutes **[1]**.

4.9.1. Talent placing the treated plate back into the humidified incubator set at 37 degrees Celsius with 5 percent carbon dioxide.

4.10. After incubation, gently add 500 microliters of culture medium to each well along the wall of the plate **[1]**. Incubate the gels again in the humidified incubator for 24 hours **[2]**.

4.10.1. Talent tilting the plate and slowly adding 500 microliters of medium down the side of each well.

4.10.2. Talent placing the plate into the incubator and setting a timer for 24 hours.

5. Washing the Gels, Imaging and Analysis

5.1. Tilt the plate and aspirate the culture medium from each well **[1]**. Then, gently add 500 microliters of PBS to each well along the walls of the plate **[2]**.

5.1.1. Talent tilting the well plate and aspirating the culture medium from each well.

5.1.2. Talent gently adding 500 microliters of PBS down the wall of each well.

5.2. Incubate the gels in a humidified incubator at 37 degrees Celsius with 5 percent carbon dioxide for 5 minutes **[1]**. Then, aspirate the PBS from each well **[2]**.

5.2.1. Talent placing the plate into the humidified incubator set at 37 degrees Celsius and 5 percent carbon dioxide.

5.2.2. Talent aspirating the PBS from the wells.

5.3. Gently add 500 microliters of PBS to each well by allowing it to slide down the wall of the plate **[1]**. Gently swirl the plate **[2]**, then tilt it and aspirate the PBS from each well **[3]**.

5.3.1. Talent slowly adding 500 microliters of PBS into the wells.

5.3.2. Talent gently swirling the plate to mix the PBS across the gel surface.

5.3.3. Talent tilting the plate again and aspirating the PBS from the wells.

5.4. Now, add 100 microliters of fresh PBS to each well to keep the gels hydrated for imaging **[1]**. Then, image the plate using standard fluorescent microscopy techniques **[2]**.

5.4.1. Talent pipetting 100 microliters of fresh PBS into each well.

5.4.2. Talent placing the sample under a fluorescent microscope.

5.5. After imaging, add 500 microliters of culture medium to each well of the plate **[1]** and incubate for 24 hours **[2-TXT]**. Repeat the full imaging and recovery workflow for each designated timepoint **[3]**.

5.5.1. Talent carefully adding 500 microliters of culture medium to each well.

5.5.2. Talent placing the plate back into the incubator. **TXT: 37 °C; 5% CO_2**

5.5.3. Talent looking at the imager's screen.

5.6. After creating a computational model, use the microscope software to measure the diameter of both the outer and inner edges of the torus-shaped region along the vertical and horizontal axes **[1]**. Average the outer and inner diameters respectively and divide by two to calculate the corresponding radii **[2]**.

5.6.1. SCREEN: 68494_Shot-5.6.1_Take-1.mp4 00:12-00:45.

5.6.2. SCREEN: 68494_Shot-5.6.2_Take-1.mp4 00:06-00:22

5.7. Finally, using the lookup table created previously, derive the electric field intensity at the measured radii **[1]**.

5.7.1. SCREEN: 68494_Shot-5.7.1_Take-1.mp4 00:00-00:15

Results

6. Results

6.1. The outer radius of the transfected region was used to quantify the reversible electroporation or RE threshold by correlating it with electric field intensities from a computational model **[1]**, while the inner radius was used to determine the irreversible electroporation or IRE threshold **[2]**.

6.1.1. LAB MEDIA: Figure 2B. *Video editor: Highlight the curve*.

6.1.2. LAB MEDIA: Figure 3A–C.

6.2. All three bipolar microsecond pulse protocols resulted in torus-shaped transfection regions, with clearly visible RE and IRE boundaries **[1]**.

6.2.1. LAB MEDIA: Figure 3A–C. *Video editor: Emphasize the white and red rings*.

6.3. Among the tested waveforms, the 2-1-1 burst-balanced waveform generated the highest IRE threshold **[1]**, while the 2-1-1 unbalanced waveform showed the lowest **[2]**.

6.3.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the red bar labeled "2-1-1 Burst-Balanced Waveform," which is taller than the others*.

6.3.2. LAB MEDIA: Figure 3D. *Video editor: Highlight the red bar labeled "2-1-1 Unbalanced Waveform," which is shorter than the others*.

6.4. A standard monopolar electroporation protocol using 420 volts resulted in a circular transfection region with an RE threshold of 642 volts per centimeter but failed to produce cell death, preventing IRE threshold determination **[1]**.

6.4.1. LAB MEDIA: Figure 4A. *Video editor: Highlight the white circle in the center of the gel*.

6.5. Tissue deformation over time due to gel degradation caused the transfected regions to lose their circular shape, making accurate RE and IRE quantification difficult **[1]**.

6.5.1. LAB MEDIA: Figure 4B. *Video editor: Emphasize the dotted outlines in white and red*.

6.6. Misalignment of the ring and pin electrodes with the bottom of the well also produced

asymmetrical, non-circular transfection patterns, complicating threshold measurement **[1]**.

6.6.1. LAB MEDIA: Figure 4C. *Video editor: Highlight the white and red circular outlines*

Pronunciation Guide:

1. Electroporation

Pronunciation link: https://www.merriam-webster.com/dictionary/electroporation
IPA (US): /ɪˌlɛktr.oʊ.pəˈreɪ.ʃən/
Phonetic spelling: ih-LEK-troh-puh-RAY-shuhn

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2. Macromolecule

Pronunciation link: https://www.merriam-webster.com/dictionary/macromolecule
IPA (US): /ˌmækroʊˈmɑːlɪˌkjul/
Phonetic spelling: MAK-roh-MAH-lih-kyool

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3. Transient

Pronunciation link: https://www.merriam-webster.com/dictionary/transient
IPA (US): /ˈtræn·zi·ənt/
Phonetic spelling: TRAN-zee-uhnt

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4. Ablation

Pronunciation link: https://www.merriam-webster.com/dictionary/ablation
IPA (US): /əˈbleɪʃən/
Phonetic spelling: uh-BLAY-shuhn

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5. Polymerization

Pronunciation link: https://www.merriam-webster.com/dictionary/polymerization
IPA (US): /ˌpɑl·ə·mə·rəˈzeɪ·ʃən/
Phonetic spelling: pol-uh-muh-ruh-ZAY-shuhn

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6. Humidified

Pronunciation link: https://www.merriam-webster.com/dictionary/humidified
IPA (US): /ˈhyu·mə·dəˌfaɪd/
Phonetic spelling: HYOO-muh-duh-fyd

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7. Torus

Pronunciation link: https://www.merriam-webster.com/dictionary/torus
IPA (US): /ˈtɔr·əs/

Phonetic spelling: TOR-uhs

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8. Irreversible

Pronunciation link: https://www.merriam-webster.com/dictionary/irreversible
IPA (US): /ˌɪr·ɪˈvɜr·sə·bəl/
Phonetic spelling: ih-rih-VUR-suh-buhl

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9. Biophysical

Pronunciation link: https://www.merriam-webster.com/dictionary/biophysical
IPA (US): /ˌbaɪ.əˈfɪz·ɪ·kəl/
Phonetic spelling: bye-uh-FIZ-ih-kuhl

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10. Fluorescent

Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescent
IPA (US): /flʊˈrɛs·ənt/
Phonetic spelling: flu-RESS-uhnt