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Title: Revealing Electromechanical Control of Tissue Homeostasis Using a Two-Layer Microfluidic Device

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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? **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**

Current Protocol Length
Number of Steps: 17
Number of Shots: 43

Introduction

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

1.1. **Thuan Beng Saw:** We apply controlled electric fields to study how tissues respond to electrical cues, focusing on how currents or fields regulate cell adhesion, proliferation and extrusion impacting tissue homeostasis.

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 significant findings have you established in your field?

1.2. **Xiaolu Jiang:** We discovered that the direction of physiologically-relevant currents dictates tissue states: apical-to-basal fields induces a proliferative phenotype, while basal-to-apical fields promotes cell extrusion, allowing modulation of overall cell density.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.5.1*

What research gap are you addressing with your protocol?

1.3. **Thuan Beng Saw:** While quantitative bioelectric studies have long focused on migration via galvanotaxis, tissue homeostasis remained inaccessible. Our system now enables direct investigation of how physiological currents regulate tissue organization and maintenance.

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

What advantage does your protocol offer compared to other techniques?

1.4. **Xiaolu Jiang:** Our protocol integrates microfluidics, electrically stimulation of tissues, traction force microscopy, and live imaging to study electromechanical control of tissue homeostasis.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2*

What new scientific questions have your results paved the way for?

1.5. **Thuan Beng Saw:** Our work revealed core electromechanical coupling rules. With new tools, we can now explore how native bioelectric patterns emerge and regulate tissues and if we can control complex tissue behavior by tuning these electromechanical cues.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.5.1*

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

Protocol

2. Chip Fabrication and Device Assembly

Demonstrator: Feng Feng

2.1. To begin, place a clean Layer 1 polydimethylsiloxane or PDMS mold face-up on a clean plastic dish cap **[1-TXT]** and add 500 microliters of liquid ultraviolet-curable adhesive slowly on the mold **[2]**. Using a spatula, carefully wet all the protruding structures and remove any air bubbles formed during the wetting process **[3]**.

2.1.1. WIDE: Talent placing a clean Layer 1 PDMS mold onto a plastic dish cap. **TXT: Cap:**

Diameter: 60 mm

Height 15 mm

2.1.2. Talent adding liquid ultraviolet-curable adhesive onto the mold.

2.1.3. Talent removing air bubbles from the adhesive using the spatula.

2.2. Gently press a coverslip onto the PDMS mold **[1]** and use a laboratory wiper to remove any excess ultraviolet-curable adhesive, ensuring the top side of the coverslip remains clean and flat **[2]**.

2.2.1. Talent pressing the coverslip onto the adhesive-coated PDMS mold.

2.2.2. Talent cleaning off excess adhesive from the top of the coverslip using laboratory wipers.

2.3. Cure the structure partially under uniformly illuminated ultraviolet light with a wavelength between 363 and 370 nanometers **[1-TXT]**. Wipe off any marks using 75 percent ethanol **[2]**. Then, hold the structure firmly on a flat surface **[3]** and slowly peel the PDMS mold from the cured Layer 1 **[4]**.

2.3.1. Talent placing the assembly under UV light. **TXT: UV light intensity: 224 mW/cm²**

2.3.2. Talent wiping marks off the structure with 75 percent ethanol.

2.3.3. Talent placing the mold on a flat surface.

2.3.4. Talent peeling the PDMS mold from the cured Layer 1 while holding it flat.

2.4. Then, place a clean Layer 2 PDMS mold face-down on a flat PDMS slab that is thicker than 2.5 millimeters **[1]**. Gently tap the mold features from above using tweezers **[2]**. Observe the interface beneath the features for darkening or optical contrast, which confirms proper contact **[3]**.

2.4.1. Talent placing the PDMS mold face-down on the flat PDMS slab.

2.4.2. Talent tapping the features on the mold with tweezers.

2.4.3. Talent pointing to the interface showing contrast beneath features.

2.5. Now, fill the space between the PDMS mold and the slab with liquid ultraviolet-curable adhesive by capillary action **[1]**. Partially cure the structure under ultraviolet light for 10 seconds as demonstrated earlier **[2]**.

2.5.1. Talent applying adhesive near the interface and letting it fill the gap via capillary action.

2.5.2. Talent placing the structure under UV light for partial curing.

2.6. Use soft-padded tweezers to gently peel the composite ultraviolet-curable adhesive layer and PDMS mold from the PDMS slab **[1]**. If necessary, trim the excess edges of the cured adhesive layer with sharp scissors **[2]**.

2.6.1. Talent peeling the composite layer and mold using padded tweezers.

2.6.2. Talent trimming the edges of the cured layer with scissors.

2.7. Next, place Layer 1 attached to a rectangular glass coverslip on a flat surface **[1]**. Align Layer 2, still attached to its PDMS mold, with Layer 1 **[2]** and press both layers together firmly until shadows appear beneath the features **[3]**.

2.7.1. Talent placing Layer 1 on a rectangular coverslip on a work surface.

2.7.2. Talent aligning Layer 2 over Layer 1.

2.7.3. Talent pressing down to create visible shadows under the features.

2.8. Cure the aligned structure under ultraviolet light for 10 seconds to bond Layers 1 and 2 into a two-layer microfluidic chip **[1]**. Then, gently peel the PDMS mold from the bonded chip **[2]**.

2.8.1. Talent placing the assembly under UV light for bonding.

2.8.2. Talent peeling the PDMS mold from the microfluidic chip.

2.9. Immediately pipette 10 microliters of polyacrylamide gel precursor solution onto the

middle of the tissue region, directly above the slit in Layer 2 **[1]**. Gently press a round glass coverslip, 10 millimeters in diameter, onto the droplet to form a flat gel **[2]**. Observe the gel solution flow into Layer 1 through the slit and stop at the row of barrier pillars **[3]**.

2.9.1. Talent pipetting gel precursor onto the slit area of the chip.

2.9.2. Talent pressing a round coverslip onto the solution to spread it.

2.9.3. Close-up of the gel flowing through the slit and halting at barrier pillars.

2.10. Immediately cure the assembled microfluidic chip under ultraviolet light for 5 minutes **[1]**. Then, leave the chip undisturbed for approximately 1 hour to allow the polyacrylamide gel to fully solidify **[2]**.

2.10.1. Talent placing the chip under UV light for full curing.

2.10.2. Shot of the chip lying undisturbed.

2.11. Next, incubate the microfluidic chip in 0.1 molar HEPES buffer at pH 7.4 for at least 1 hour to hydrate the gel and reduce adhesion between the gel and the coverslip **[1]**. Then, gently use sharp tweezers to remove the coverslip from the gel surface **[2]**.

2.11.1. Talent immersing the chip in HEPES buffer.

2.11.2. Talent removing the coverslip from the gel using sharp tweezers.

Added step

2.12. Place a medical-grade polycarbonate cartridge on a flat surface, with the bottom face up, and align a dried chip, face down, with the cartridge **[1]**. Pipette UV-curable adhesive in the crevices between the chip and cartridge, filling them via capillary effect, and cure it under UV light **[2-TXT]**.

Added shot : Talent bonding the chip to cartridge.

Added shot: Talent adding the adhesive and placing the composite under UV light. **TXT: Cure with UV light (5 min, 224 mW/cm²)**

3. Polyacrylamide (PA) Gel Functionalization with ECM Protein

3.1. Sterilize the fabricated microfluidic devices by placing them under ultraviolet light at 200 to 280 nanometers inside a biosafety cabinet **[1]**.

3.1.1. Talent placing multiple chips in a biosafety cabinet and switching on the UV light for sterilization.

3.2. Prepare the required volume of 50 micrograms per milliliter Collagen I *(1)* working solution in 1× DPBS on ice **[1]**.

3.2.1. Talent pipetting Collagen I into cold 1× DPBS and gently mixing the solution over ice.

3.3. On ice, prepare the Sulfo-SANPAH *(San-puh)* working solution by diluting 2 microliters of Sulfo-SANPAH stock solution, previously dissolved in anhydrous DMSO **[1]**, in 80 microliters of cold 0.1 molar HEPES buffer at pH 7.4, stored at 4 degrees Celsius **[2]**.

3.3.1. Talent aspirating 2 microliters of SS stock into a micropipette.

3.3.2. Shot of adding SS to a tube containing 80 microliters of cold HEPES and gently mixing it over an ice tray.

3.4. Dab the edges of the polyacrylamide gel with laboratory wipes to dry it inside the device **[1]**. Pipette 80 microliters of Sulfo-SANPAH working solution onto the gel surface, ensuring full immersion **[2]** and place the chip under ultraviolet light for 5 minutes to activate the Sulfo-SANPAH **[3-TXT]**. Rinse the gel three times with cold 0.1 molar HEPES buffer at pH 7.4 **[4]**.

3.4.1. Talent using laboratory wipes to dry the gel by dabbing the edges.

3.4.2. Talent pipetting SS working solution over the gel surface until fully covered.

3.4.3. Talent placing the chip under UV light for 5 minutes. **TXT: UV light intensity: 24.5 mW/cm^2**

3.4.4. Talent rinsing the gel three times with cold HEPES buffer.

3.5. Repeat the Sulfo-SANPAH treatment by pipetting 80 microliters of SS working solution over the gel **[1]** and irradiating it under ultraviolet light for 5 minutes **[2]**. This time, rinse the gel three times with cold 1× DPBS **[3]**.

3.5.1. Talent applying SS solution again on the gel.

3.5.2. Talent turning on the UV light switch.

3.5.3. Talent adding cold 1× DPBS onto the gel.

3.6. Finally, pipette 100 microliters of Collagen I solution onto the polyacrylamide gel,

ensuring complete coverage and leave it for 1 hour **[1]**. Rinse off unattached collagen with 1× DPBS three times **[2]**, then keep the gel immersed in 1× DPBS until further use **[3-TXT]**.

3.6.1. Talent applying Collagen I solution over the entire gel surface.

~~3.6.2. Talent leaving the chip at room temperature on the bench.~~**NOTE**: Not filmed, VO merged

3.6.3. Talent adding the gel with 1× DPBS.

3.6.4. Talent dipping the gel in DPBS. **TXT: Use the fabricated device for live cell experiments**

4. Connection of Devices to Electrode Chambers and Live-Cell Imaging

4.1. Attach devices to the inserts using screws **[1-TXT]**. Then, secure up to four device-insert assemblies to the holder designed for mounting on a microscope stage **[2-TXT]**.

4.1.1. Talent fastening one device to an insert with screws. **TXT: Screw type: M3 X 10 mm**

4.1.2. Shot of the fully set four device-insert assemblies with the holder. **TXT: Screw type: M3 X 5 mm**

4.2. Add 2 milliliters of culture medium to each main well of the devices and through the waste removal channels to flush out and replace the 1X DPBS **[1]**.

4.2.1. Talent pipette 2 ml DMEM++ into the main wells and through the waste removal channels.

4.3. Then, insert the platinum electrodes into the tube containing 45 milliliters of medium, ensuring they are fully submerged in the medium **[1]**.

4.3.1. Talent threading electrodes and tubing through the cap and submerging them.

4.4. Pull culture medium through the Tygon tubing with a syringe and clip it tight **[1]**. Connect one end of the medium-filled tubing to the electrode chamber and the other end to the device **[2]**.

4.4.1. Talent putting one end of tubing into medium and aspirating at the other end with syringe till tubing fills with medium.

4.4.2. Talent connect one end of the medium-filled tubing to the electrode chamber

and the other end to the device.

4.5. Now, prepare a cell suspension by following the trypsinization or cell suspension preparation recommendations and seed 0.1 million cells into the main well **[1-TXT]**~~. Let the cells sediment for 15 minutes before slowly transferring the whole setup into a tissue culture incubator **[2]**~~.

4.5.1. Talent pipetting cell resuspension into the main well of chip. **TXT: Let the cells sediment for 15 min**

~~4.5.2. Talent mounting the whole setup into incubator.~~ **NOTE**: Not filmed, VO moved as on screen text

4.6. Mount the setup onto the microscope, with the electrode chambers on the sides **[1]** and connect the source meter to the electrode chamber **[2]**.

4.6.1. Talent placing a setup on to microscope stage, then mounting the electrode chamber into cage.

4.6.2. Talent connect source meter to electrode.

4.7. Use 3 devices with identical channel resistance. Assign one as a control group, apply apical-to-basal current to the second device and basal-to-apical current to the third device **[1]**. Source an electric signal under current-clamp mode and supply a total current of 20 microamperes **[2]**.

4.7.1. Shot of 3 chip lying in microscope stage.

4.7.2. Talent selecting current-clamp and supplying a total current of 20 uA mode on source meter.

4.8. Choose 20x objective lens for whole-tissue imaging with an epifluorescence microscope **[1]**.

4.8.1. Talent clicking on 20x objective lens on the screen.

4.9. Finally, choose channel 561 for red fluorescence and bright-field channels **[1]**. Select 1 1-hour time interval during long-time imaging **[2]**.

4.9.1. Talent choosing 5 rows by 5 columns, the middle row covering the slit, while the others outside of the slit.

4.9.2. Talent choosing bright-field to show tissue. Then choosing channel 561 to show beads.

Results

5. Results

5.1. Electric stimulation was applied perpendicular to the cells to disrupt transepithelial potential to study its role in tissue behavior under different current directions **[1]**. Under phase-contrast imaging, apical-to-basal or AtB *(A-T-B)* currents caused cell junctions to brighten within 10 minutes of application **[2]**, while control and basal-to-apical BtA *(B-T-A)* conditions showed low contrast and flat apical surfaces **[3]**.

5.1.1. LAB MEDIA: Figure 3

5.1.2. LAB MEDIA: Figure 3A, C, B. *Video editor: Highlight the "AtB" images and curve in B*.

5.1.3. LAB MEDIA: Figure 3A, C, B. *Video editor: Highlight the "Control" and "BtA" images in A, C and curve in B*.

5.2. AtB currents produced an outward convexity at the apical cell surface **[1]**.

5.2.1. LAB MEDIA: Figure 3C. *Video editor: Zoom in on the AtB image*.

5.3. Gel deformation was strongly dependent on the direction of current. AtB currents pulled the gel upward **[1]**, while BtA currents compressed the gel downward **[2]**.

5.3.1. LAB MEDIA: Figure 3D. *Video editor: Highlight the AtB panel under gel deformation*.

5.3.2. LAB MEDIA: Figure 3D. *Video editor: Highlight the BtA panel under gel deformation*.

5.4. Junctional E-cadherin intensity increased under BtA currents **[1]**, but significantly decreased under AtB currents **[2]** compared to control **[3]**.

5.4.1. LAB MEDIA: Figure 4A B C. *Video editor: Highlight BtA images in A, C and curve in B*.

5.4.2. LAB MEDIA: Figure 4A B C. *Video editor: Highlight AtB images in A, C and curve in B*.

5.4.3. LAB MEDIA: Figure 4 A B C. *Video editor: Focus on the "CONTROL" images in A, C and curve in B*.

5.5. Junctional actin intensity decreased under AtB currents **[1]**, but did not significantly increase under BtA currents **[2]** relative to control **[3]**.

5.5.1. LAB MEDIA: Figure 4D E F. *Video editor: Highlight AtB images in D, F and curve in E*.

5.5.2. LAB MEDIA: Figure 4D E F. *Video editor: Highlight BtA images in D, F and curve in E*.

5.5.3. LAB MEDIA: Figure 4D E F. *Video editor: Highlight Control images in D, F and curve in E*

5.6. AtB currents triggered multicellular live cell extrusions across the monolayer **[1]**, while BtA currents preserved monolayer structure similar to the control **[2]**.

5.6.1. LAB MEDIA: Figure 5A and B. *Video editor: Highlight the white patch clusters in the AtB panel*.

5.6.2. LAB MEDIA: Figure 5A,B. *Video editor: Emphasize BtA and Control layers*.

5.7. Spatial heterogeneity in cell distribution was significantly higher under AtB currents **[1]**, but remained low and uniform under BtA and control conditions **[2]**.

5.7.1. LAB MEDIA: Figure 5C. *Video editor: Emphasize the taller scatter points for AtB in the RUDM graph*.

5.7.2. LAB MEDIA: Figure 5C. *Video editor: Highlight the dense clustering of dots for BtA and Control*.

5.8. Cell proliferation rates increased under AtB stimulation, with higher division rates over time **[1]**. In contrast, BtA currents induced more cell death events **[2]**.

5.8.1. LAB MEDIA: Figure 5D.

5.8.2. LAB MEDIA: Figure 5E. *Video editor: Highlight the elevated data points for BtA*

- **Polydimethylsiloxane (PDMS)**

Pronunciation link:
https://www.merriam-webster.com/dictionary/polydimethylsiloxane
IPA: /ˌpɑːliˌdaɪˌmɛθɪlˈsaɪˌlɑkseɪn/
Phonetic Spelling: pol-ee-dy-meth-il-sy-lok-sane

- **Microliter**

Pronunciation link:
https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪkroʊˌliːtər/
Phonetic Spelling: my-kroh-lee-ter

- **Ultraviolet**

Pronunciation link:
https://www.merriam-webster.com/dictionary/ultraviolet
IPA: /ˌʌltrəˈvaɪələt/
Phonetic Spelling: uhl-truh-vy-uh-let

- **Coverslip**

Pronunciation link:
https://www.merriam-webster.com/dictionary/coverslip
IPA: /ˈkʌvərˌslɪp/
Phonetic Spelling: kuh-ver-slip

- **Ethanol**

Pronunciation link:
https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθəˌnɔl/
Phonetic Spelling: eth-uh-nawl

- **Nanometer**

Pronunciation link:
https://www.merriam-webster.com/dictionary/nanometer
IPA: /ˈnænəˌmiːtər/
Phonetic Spelling: nan-uh-mee-ter

- **Capillary**

Pronunciation link:
https://www.merriam-webster.com/dictionary/capillary
IPA: /ˈkæpəˌlɛri/
Phonetic Spelling: cap-uh-lair-ee

- **Microfluidic**

No confirmed link found
IPA: /ˌmaɪkroʊfluːˈɪdɪk/
Phonetic Spelling: my-kroh-floo-id-ik

- **Polyacrylamide**

Pronunciation link:
https://www.merriam-webster.com/dictionary/polyacrylamide

IPA: /ˌpɑːliˈækrɪləˌmaɪd/
Phonetic Spelling: pol-ee-ak-ri-luh-mide

- **HEPES**

No confirmed link found
IPA: /ˈhɛpiːz/
Phonetic Spelling: hep-eez

- **Polycarbonate**

Pronunciation link:
https://www.merriam-webster.com/dictionary/polycarbonate
IPA: /ˌpɑːliˈkɑːrbəˌneɪt/
Phonetic Spelling: pol-ee-kar-buh-nate

- **Cartridge**

Pronunciation link:
https://www.merriam-webster.com/dictionary/cartridge
IPA: /ˈkɑrtrɪdʒ/
Phonetic Spelling: kar-trij

- **Collagen**

Pronunciation link:
https://www.merriam-webster.com/dictionary/collagen
IPA: /ˈkɑlədʒən/
Phonetic Spelling: kol-uh-jen

- **DPBS** (Dulbecco's Phosphate-Buffered Saline)

No confirmed link found
IPA: /ˌdiːpiːbiːˈɛs/
Phonetic Spelling: D-P-B-S

- **Sulfo-SANPAH**

No confirmed link found
IPA: /ˈsʌlfoʊ ˈsænˌpɑː/
Phonetic Spelling: sul-foh san-pah

- **Anhydrous**

Pronunciation link:
https://www.merriam-webster.com/dictionary/anhydrous
IPA: /ænˈhaɪdrəs/
Phonetic Spelling: an-hy-drus

- **DMSO (Dimethyl sulfoxide)**

Pronunciation link:
https://www.merriam-webster.com/dictionary/dimethyl%20sulfoxide

IPA: /ˌdaɪˌmɛθəlˈsʌlfɑkˌsaɪd/
Phonetic Spelling: dye-meth-il-sul-fok-side

- **Biosafety**

Pronunciation link:
https://www.merriam-webster.com/dictionary/biosafety
IPA: /ˌbaɪoʊˈseɪfti/
Phonetic Spelling: bye-oh-safe-tee

- **Transepithelial**

No confirmed link found
IPA: /ˌtrænsˌɛpɪˈθiːliəl/
Phonetic Spelling: trans-ep-ih-thee-lee-uhl

- **Epifluorescence**

No confirmed link found
IPA: /ˌɛpiˌflʊˈrɛsəns/
Phonetic Spelling: eh-pee-flor-ess-ens

- **Cadherin**

Pronunciation link:
https://www.merriam-webster.com/dictionary/cadherin
IPA: /kædˈhɪrən/
Phonetic Spelling: kad-hear-in

- **Actin**

Pronunciation link:
https://www.merriam-webster.com/dictionary/actin
IPA: /ˈæktən/
Phonetic Spelling: ak-tin