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Title: Isolation and Time-Lapse Imaging of Primary Mouse Embryonic Palatal Mesenchyme Cells to Analyze Collective Movement Attributes

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Author Questionnaire

1. Microscopy: Does your protocol demonstrate the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or similar? **Y**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Interview statements: Considering the Covid-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

☒ Interviewees wear masks until the videographer steps away (≥6 ft/2 m) and begins filming. The interviewee then removes the mask for line delivery only. When the shot is acquired, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Protocol Length
Number of Shots: **42**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Jeremy Goering**: This protocol allows investigators in the craniofacial field to quantitatively compare collective movement attributes in control and mutant cells as a means to understand mesenchymal remodeling during palatal shelf elevation **[1]**.

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

REQUIRED:

1.2. **Jeremy Goering**: The use of primary mouse embryonic palatal mesenchyme cells and time-lapse imaging provide an accessible proxy method for assessing palatal shelf elevation dynamics **[1]**.

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

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kansas Medical Center.

Protocol

2. Embryonic Palatal Shelf Dissection

2.1. To harvest palatal shelves from a mouse embryo, use a sterilized perforated spoon to place an embryonic day 13.5 embryo into a sterile 10-centimeter dish filled with sterile MEPM (M-E-P-M) culture medium under a stereomicroscope **[1-TXT]**.

2.1.1. WIDE: Talent placing embryo into dish, with microscope visible in frame *Videographer: More Talent than mouse in shot* **TEXT: Dissection demonstrated in PBS for visual clarity** Text was edited down from authors suggestion, in case they ask why the onscreen text as shortened

2.2. ~~Decapitate the embryo right below the jaw line using sterile scissors.~~ **~~[1]~~** After decapitation, insert one tip of a sterilized fine number 5 forceps into the mouth just inside the cheek **~~[2]~~** and push the tip of the forceps until it exits the back of the skull **[2]**.

~~2.2.1. SCOPE: Embryo decapitation~~ Authors provided this shot, obviously do not use this

2.2.2. LAB MEDIA: 2.2.2-2.2.3

~~2.2.3. SCOPE: Tips being pushed~~

2.3. Orient the forceps so that the other arm hovers just over the ear canal **~~[1]~~** before pinching the forceps shut to cut the tissue **[1-TXT]**. ~~If necessary, run another pair of fine forceps along the seam of the closed forceps to cut through any tissue that was not completely severed by the pinch~~ **~~[3]~~**.

2.3.1. LAB MEDIA: 2.3.1-2.3.3: 00:12-00:23 **TEXT: Run fine forceps along closed forceps seam to completely sever tissue as necessary**

~~2.3.2. SCOPE: Forceps being closed~~ ~~*Videographer: Important step*~~

~~2.3.3. SCOPE: Forceps being run along closed forceps seam~~ ~~*Videographer: Important step*~~

2.4. Repeat the incision on the other side of the head ~~[1]~~, continuing the pinch-cut procedure until the lower jaw, tongue, and inferior portion of the skull have been removed **[1]**, exposing the palatal shelves **[2]**.

2.4.1. LAB MEDIA: 2.4.1-2.4.2

~~2.4.2. SCOPE: Tissue being cut~~

2.4.3. LAB MEDIA: 2.4.3: 00:06-00:12

2.5. With the head placed on its side, position the tips of a pair of small, sterile, stainless steel scissors in front of and behind the skull just about the eye level of the embryo ~~[1]~~ and cut just above the eyes to remove the cranium of the skull ~~[2]~~, creating a flat surface **[1]**.

2.5.1. LAB MEDIA: 2.5.1.-2.5.3: 00:03-00:25

~~2.5.2. SCOPE: Cut being made~~

~~2.5.3. SCOPE: Shot of flat surface~~

2.6. Next, position the head upside down with the superior aspect resting flat on the bottom of the dish ~~[1]~~ and locate the palatal shelves, which should be visible as two raised ridges on either side of a central groove in the anterior half of the head **[1]**.

2.6.1. LAB MEDIA: 2.6.1-2.6.2: 00:18-00:27

~~2.6.2. SCOPE: Shot of palatal shelves *Video Editor: please indicated palatal shelves as emphasized in Figure 1E*~~

2.7. To secure the head to the dish, insert one tip of a fine forceps through the tissue near the nasal region of the head anterior to the palatal shelves ~~[1]~~ and the other through the base of the skull posterior to the palatal shelves **[1]**.

2.7.1. LAB MEDIA: 2.7.1-2.7.2

~~2.7.2. SCOPE: Tip being inserted posterior to shelves~~

2.8. Insert both points of a second pair of very sharp fine forceps into the tissue at the base of the lateral surface of the shelf ~~**[1]**~~ and slowly and carefully pinch to cut the tissue **[1]**.

2.8.1. LAB MEDIA: 2.7.1-2.9.2: 00:14-00:25

~~2.8.2. SCOPE: Tissue being pinched/cut~~ ~~*Videographer: Important/difficult step*~~

2.9. Repeat the incision along the base of the medial surface of the shelf ~~**[1]**~~ and at both the anterior and posterior ends of the shelf to detach the shelf from its attachment to the head **[1]**.

2.9.1. LAB MEDIA: 2.9.1-2.10.1: 00:33-00:44

~~2.9.2. SCOPE: Ends being excised~~ ~~*Videographer: Difficult step*~~

2.10. Then gently lift the shelf, making additional pinches as necessary to completely free the shelf from the surrounding tissue **[1]**. ~~and place the isolated tissue in PBS **[2]**~~.

2.10.1. LAB MEDIA: 2.9.1-2.10.1: 00:44-01:01

~~2.10.2. : Tissue being placed in PBS~~ ~~*Videographer: Important step*~~

2.11. When the second palatal shelf has been removed in the same manner, use a sterile plastic bulb transfer-pipette ~~**[1]**~~ to transfer the shelves into a sterile, 1.5-milliliter microcentrifuge tube in approximately 500 microliters of PBS on ice **[2]**.

~~2.11.1. SCOPE: Tissue being aspirated~~

2.11.2. WIDE: Talent adding tissues to tube on ice ~~**TEXT: If performing dissection in medium, add 500 µL PBS to tube before adding shelves**~~ Authors recommended this onscreen text, I think we can skip it and just say “add them in PBS” but I left the text here in case they still want to add it

3. Mouse Embryonic Palatal Mesenchymal (MEPM) Cell Culture

3.1. To set up an MEPM cell culture, when all of the shelves have been collected, aspirate the PBS without disturbing the tissues **[1]** and immediately add 200 microliters of 37-degree Celsius 0.25% trypsin to each tube of palatal shelf tissue **[2]**.

3.1.1. WIDE: Talent aspirating PBS

3.1.2. Talent adding trypsin to tube(s), with trypsin container visible in frame

3.2. Use a 1000-microliter pipette to briefly pipet the tissues a few times **[1]** and incubate the tissues for 10 minutes at 37 degrees Celsius, pipetting briefly after 5 minutes **[2]**.

3.2.1. Talent pipetting tissues

3.2.2. Talent placing tube(s) at 37 °C

3.3. At the end of the incubation, pipet the tissues again **[1]** before adding 800 microliters of MEPM culture medium to each tube **[2]** and centrifuging the tubes to collect the cells **[3-TXT]**.

3.3.1. Talent pipetting tissues

3.3.2. Talent adding medium to tube(s), with medium container visible in frame

3.3.3. Talent placing tube(s) into centrifuge **TEXT: 5 min, 200 x g, RT**

3.4. After aspirating the supernatants, resuspend the pellets in 1 milliliter of fresh MEPM culture medium per tube **[1]** and plate the cells into individual wells of a 6-well tissue culture-treated plate containing 2 milliliters per well to a final total of 3 milliliters per well **[2]**.

3.4.1. Shot of pellet if visible aspirating the old media, then medium being added to tube, with medium container visible in frame. NOTE: Authors think this single step was actually filmed in 3 separate shots 1) the cell pellet, 2) old medium being aspirated, 3) fresh medium being added to the tube.

3.4.2. Talent adding cells to well(s)

3.5. Then allow the cells to adhere to the plastic surface for 12 hours in a sterile cell culture incubator **[1]**.

3.5.1. Talent placing plate into incubator

4. 2D Collective Migration Assay Setup

4.1. To set up a 2D collective migration assay, remove the top of one sterile, 2-well silicone insert to a height of approximately 1 millimeter for each sample to be analyzed **[1]** and use sterile forceps to plate the shortened, sterile 2-well inserts into the center of individual wells of a 6-well plate **[2]**.

4.1.1. WIDE: Talent cutting top of insert *Videographer: Important step*

4.1.2. Talent placing insert into well *Videographer: Important step*

4.2. Press down along all of the edges to ensure that the inserts are fully adhered to the well bottoms **[1]** and seed 300 MEPM cells/square-millimeter of the shortened silicone inserts in a total volume of 40-50 microliters of MEPM culture medium into each insert for an overnight incubation in the cell culture incubator **[2-TXT]**.

4.2.1. Edges being pressed

4.2.2. Cells being added to insert **TEXT: See text for live 2D collective migration assay imaging details**

5. Wound Repair Assay Setup

5.1. To set up a wound repair assay, use sterile forceps to place one unmodified sterile, 2-well silicone insert into the center of one well of a 6-well plate per sample **[1]** and firmly press the edges of the insert to attach the insert to the culture plate **[2]**.

5.1.1. WIDE: Talent placing insert into well

5.1.2. Insert being pressed

5.2. Then seed 1400 cells/square-millimeter in 100 microliters of MEPM culture medium into each insert **[1]** and incubate the cells for 48 hours in the cell culture medium **[2-TXT]**.

5.2.1. Talent adding cells to insert

5.2.2. Talent placing plate into incubator **TEXT: See text for wound repair assay imaging details**

Results

6. Results: Representative MEPM Imaging and Collective Movement Analysis

6.1. When performing a 2D collective migration assay **[1]**, seeding the cells in 2-well silicone inserts in a large culture dish **[2]** typically provides better cell density for imaging **[3]**.

6.1.1. LAB MEDIA: Figures 2C and 2E-2G
6.1.2. LAB MEDIA: Figures 2C and 2E-2G *Video Editor: please emphasize Figure 2C/inserts in Figure 2C*
6.1.3. LAB MEDIA: Figures 2C and 2E-2G *Video Editor: please sequentially emphasize Figures 2E-2G*

6.2. Small, 3D-printed rings placed in a 35-millimeter dish can also be used for 2D motility analysis **[1]**.

6.2.1. LAB MEDIA: Figure 2D *Video Editor: please emphasize rings in dish*

6.3. For wound-repair assays **[1]**, the cells are grown in 2-well silicone inserts until high confluence **[2]**. The inserts are then removed, and the wound is imaged until closure **[3]**.

6.3.1. LAB MEDIA: Figures 3C-3F
6.3.2. LAB MEDIA: Figure 3C-3F *Video Editor: please emphasize Figure 3C/inserts in Figure 3C*
6.3.3. LAB MEDIA: Figure 3C-3F *Video Editor: please sequentially emphasize Figures 3D-3F*

6.4. MEPM trajectories are persistent **[1]** and the direction of the cell motility is maintained for several hours **[2]**.

6.4.1. LAB MEDIA: Figure 4E
6.4.2. LAB MEDIA: Figure 4E *Video Editor: please emphasize green and red lines*

6.5. The mean displacement versus time analysis indicates that the persistence in the form of displacement is proportional to the elapsed time **[1]**.

6.5.1. LAB MEDIA: Figure 4F *Video Editor: please emphasize data lines*

6.6. Flow analysis of the motility data reveals that the co-moving MEPM cell clusters are approximately 300 microns in size **[1]**.

6.6.1. LAB MEDIA: Figures 5I and 5J

6.7. A profound motility difference between wild type **[1]** and mutant MEPM cells is also observed **[2]**.

6.7.1. LAB MEDIA: Figures 5I and 5J *Video Editor: please emphasize blue data points*
6.7.2. LAB MEDIA: Figures 5I and 5J *Video Editor: please emphasize red data points*

Conclusion

7. Conclusion Interview Statements

7.1. **Jeremy Goering**: This procedure could be used on various transgenic mouse lines and to treat MEPM cells with various biochemical agents to study their effects on cell movement **[1]**.

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

7.2. **Jeremy Goering**: We have focused on primary palatal mesenchyme cells, but the time-lapse imaging and quantitative analyses can also be used to explore the migration attributes of any motile cell type in dynamic developmental processes **[1]**.

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