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Scriptwriter Name: Shehnaz Lokhandwala
Supervisor Name: Anastasia Gomez
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Title: μ-Tongue: A Microfluidics-Based Functional Imaging Platform for the Tongue In Vivo

Authors and Affiliations:
Jisoo Han[1,2], Pyonggang Choi[3], Myunghwan Choi[3]

[1]Department of Biomedical Engineering, Sungkyunkwan University
[2]Center for Neuroscience Imaging Research, Institute for Basic Science
[3]School of Biological Sciences, Seoul National University

Corresponding Authors:
Myunghwan Choi (choim@snu.ac.kr)

Email Addresses for All Authors:
ppjisoo@skku.edu
revlils43@snu.ac.kr
choim@snu.ac.kr

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**

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?

   ☒ Interview Statements are read by JoVE's voiceover talent.

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

Current Protocol Length

Number of Steps: 28
Number of Shots: 62

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. This protocol allows observing the functions of taste cells in a natural microenvironment with neural connections and blood circulation remaining intact. Using this technique, cell-to-cell communication can be investigated in vivo.

1.1.1. *3.12.3, 3.12.4*

Ethics Title Card

1.2. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Sungkyunkwan University and Seoul National University

Protocol

2. Preparation of the Microfluidic System

2.1. To begin, fill the reservoirs of the pressurized flow perfusion system **[1]** with artificial saliva and tastants **[2]**.

2.1.1. WIDE: Talent in front of the pressurized flow system, ready to fill reservoirs.

2.1.2. Talent filling the reservoirs.

2.2. Connect the compressed air line to the regulator input **[1]** and set the air pressure in the fluidic delivery system to 30 to 50 pounds per square inch **[2]**.

2.2.1. Talent connecting the compressed air line to the regulator input.

2.2.2. Talent setting the air pressure.

2.3. Set the output pressure **[1]** of the regulator to 0.4 pounds per square inch **[2]** and check if liquid starts flowing out from the tube **[3]**.

2.3.1. Talent at the computer, monitor visible in frame. *Videographer: Obtain a few shots of talent clicking the mouse and typing on the keyboard to use as b-roll throughout the video*

2.3.2. SCREEN: 62361_screenshot_1. 0:10-0:15.

2.3.3. Liquid flowing out of the tube.

2.4. Connect the manifold from the reservoirs to the input port of the micro-tongue **[1]**. Then, connect the output port of the micro-tongue to a syringe pump **[2]** and withdraw liquid at approximately 300 microliters per minute to establish a steady state condition **[3]**.

2.4.1. Talent connecting the manifold from the reservoirs to the input port of the micro-Tongue.

2.4.2. Talent connecting the output port of the micro-tongue to a syringe pump.

2.4.3. Shot of syringe pump display showing refill rate as 300 µL/min.

2.5. Confirm that the volume of the droplet hanging under the micro-tongue remains constant **[1]** and adjust the air pressure and flow rate **[2]** to obtain the desired sample height **[3]**. *Videographer: This step is important!*

2.5.1. ECU: Shot of hanging droplet maintaining a constant volume.

2.5.2. SCREEN: 62361_screenshot_2. 0:09-0:19

2.5.3. ECU: Droplet as the pressure changes.

2.6. Once the microfluidic system has been set-up, disconnect the compressed air line **[1]** and switch off the syringe pump until the mouse has been prepared for in vivo imaging **[2]**.

2.6.1. Talent disconnecting the compressed air line.

2.6.2. Talent switching the syringe pump off.

3. Mouse Preparation for In Vivo Imaging

3.1. After administering anesthesia to the mouse **[1]**, intravenously inject TRITC-dextran through a retro-orbital route to observe blood circulation during imaging **[2]**. *Videographer: This step is important!*

3.1.1. WIDE: Talent at the workbench with anesthetized mouse in view.

3.1.2. Talent injecting TRITC-dextran into the mouse. **TEXT: 500 kDa TRITC-dextran in 2.5% W/V PBS**

3.2. With the mouse placed in a supine position, spray 70% ethanol on its head **[1]**. Use forceps to lift the head skin lightly, then use scissors to snip off approximately 7 square millimeters of skin **[2]**.

3.2.1. Talent spraying 70% ethanol on the head of the mouse.

3.2.2. Talent lifting the head skin with forceps and cutting it with scissors.

3.3. After cleaning the hair around the scalp **[1]**, remove the periosteum from under the skin **[2]** and apply an instant adhesive to the skull **[3]**. Then, attach the customized head fixer **[4].**

3.3.1. Talent cleaning hair.

3.3.2. Talent removing the periosteum.

3.3.3. Talent applying adhesive.

3.3.4. Talent attaching the customized head fixer.

3.4. Once the instant adhesive is hardened, apply dental glue around the head fixer **[1]** and solidify it by illumination with a blue light **[2].**

3.4.1. Talent applying dental glue.

3.4.2. Talent using a blue light to solidify the glue.

3.5. Use an instant adhesive to glue the lower lip of the mouse to the bottom unit of the micro-tongue **[1]**. Then, place the mouse on the mouse preparation board **[2]** and secure the bottom unit of the micro-tongue to the hold posts by aligning the holes at the edge of the micro-tongue with the posts **[3]**.

3.5.1. Talent gluing the lower lip of mouse to the bottom unit of the micro-tongue.

3.5.2. Talent placing the mouse on the board.

3.5.3. Talent securing the bottom unit of the micro-tongue to the hold posts.

3.6. Tighten the mouse head fixer to the head fixer holder at the board **[1]** and adjust the distance between the mouse head and the device **[2]**. Then, using the head fixer holder, rotate the mouse head smoothly by approximately 45 degrees **[3]**.

3.6.1. Talent tightening the mouse head fixer to the head fixer holder.

3.6.2. Talent adjusting the distance between the mouse head and the device.

3.6.3. Talent rotating the mouse head.

3.7. After securing the mouse to the board, use plastic tweezers to draw its tongue gently, **[1]** then using instant adhesive, attach the ventral side of the tongue to the upper side of the bottom unit of the micro-tongue **[2]**. *Videographer: This step is important!*

3.7.1. Talent drawing the tongue out with tweezers.

3.7.2. Talent attaching the tongue to the bottom unit of the micro-tongue.

3.8. To keep the tongue moist, wipe the surface with a wet cotton swab **[1]**, then place a piece of tissue soaked in artificial saliva on the exposed surface of the tongue **[2]**. *Videographer: This step is important!*

3.8.1. Talent wiping the tongue with a cotton swab.

3.8.2. Talent placing a soaked paper on the tongue.

3.9. Place the curved washers on the posts holding the bottom part of the micro-tongue **[1]**, then move the mouse preparation to the microscope stage **[2]**.

3.9.1. Talent placing the curved washers on the posts.

3.9.2. Talent moving the mouse preparation to the microscope.

3.10. Position the exposed tongue of the mouse under the approximate center of the microscope objective area, making sure not to deviate from the dynamic range of the stage **[1]**, then tighten the screws to firmly attach the mouse board on the stage **[2]**

3.10.1. Talent positioning the tongue under the microscope objective.

3.10.2. Talent tightening screws.

3.11. Place a heating pad under the mouse to maintain its body temperature between 36.5 and 37.5 degrees Celsius **[1]**.

3.11.1. Talent placing the heating pad under the mouse.

3.12. To prevent liquid from entering the mouse trachea, place a thin piece of twisted paper inside the mouse's mouth **[1]**. Then, remove the wet tissue placed on the surface of the tongue **[2]** and place the prepared micro-tongue on the mouse tongue **[3]** such that the surface of the tongue is visible through the imaging window **[4].**

3.12.1. Talent placing twisted paper inside the mouse's mouth. *Videographer: This step is important!*

3.12.2. Talent removing the wet tissue.

3.12.3. Talent placing the micro-tongue on the mouse tongue.

3.12.4. ECU: Tongue surface in the imaging window.

3.13. Secure the micro-tongue by gently screwing both ends with minimal compressive pressure **[1]**.

3.13.1. Talent securing the micro-tongue.

4. Imaging Acquisition

4.1. To begin acquiring images **[1],** open the microscope software **[2]**, then turn on the 920-nanometer two-photon laser **[3]**.

4.1.1. WIDE: Talent sitting at the computer, monitor visible in frame

4.1.2. SCREEN: 62361_screenshot_3. 0:03-0:05; 0:11-0:14; then skip to 0:24. *Video Editor: Speed up, as necessary*

4.1.3. Talent turning on the laser.

4.2. Mount the water-immersion objective on the microscope **[1-TXT]**. Then, drop distilled water on the imaging window of the micro-tongue **[2]** and immerse the objective **[3]**.

4.2.1. Talent mounting the water-immersion objective. **TEXT: 16x, NA 0.80 or 25x, NA 1.1**

4.2.2. Talent dropping water on the imaging window.

4.2.3. Talent immersing the objective.

4.3. In camera mode, illuminate the surface of the tongue by turning on the blue light from a mercury lamp **[1]**.

4.3.1. Talent turning on the blue light.

4.4. To find the approximate focal plane **[1]**, adjust the Z-axis and search for an auto fluorescent signal from the filiform papillae **[2]**. Then, using the X and Y adjustment knob, locate a taste bud **[3]**.

4.4.1. Talent at the computer, in the camera mode, monitor in frame.

4.4.2. SCREEN: 62361_screenshot_4 (4.4.1). 0:02-0:08. *Video Editor: Zoom in on the software window and emphasize the changing Z-axis value (pointed at by the cursor) while the image comes into focus*

4.4.3. SCREEN: 62361_screenshot_4 (4.4.2). 0:05-0:17. *Video Editor: Speed up, as necessary*

4.5. Switch to the multiphoton mode **[1]**, then set the excitation wavelength, emission filter set, scan mode, and frame size for image acquisition **[2]**.

4.5.1. SCREEN: 62361_screenshot_5 (4.5.1). 0:03-0:07; then skip to 0:10-0:11.

4.5.2. SCREEN: 62361_screenshot_5 (4.5.2). 0:03-0:23; 0:28-0:30; 0:34-0:51. *Video Editor: Speed up, as necessary*

4.6. With a taste bud at the center of the imaging window, locate the blood vessels surrounding the taste bud at about two-thirds the height of the taste bud and

visualize the blood circulation. If the blood flow is clogged, slightly loosen the fixing screws to resume blood flow **[1].**

4.6.1. SCREEN: 62361_screenshot_6. 0:02-0:20. *Video Editor: Emphasize the Z-axis value changing between 0:03-0:08.*

4.7. Adjust the Z-axis to find the Z-plane of a taste bud containing an adequate number of taste cells **[1]**, then proceed with calcium imaging at 2 to 6 Hertz for 80 seconds **[2]**.

4.7.1. SCREEN: 62361_screenshot_7. 0:03-0:08. *Video Editor: Emphasize the changing Z-axis value.*

4.7.2. SCREEN: 62361_screenshot_7. 0:14-0:28.

4.8. After imaging starts, switch on the reservoir of the fluidic system to provide a taste solution for 20 seconds **[1]**. After 20 seconds of taste stimulation, switch the reservoir back to artificial saliva **[2]**.

4.8.1. Talent switching on the reservoir to provide taste solution.

4.8.2. Talent switching reservoir back to artificial saliva.

4.9. During imaging, wait for about 3 to 4 minutes between sessions, consistently providing artificial saliva to keep the tongue moist and wash away the tastant remaining from the previous session **[1].**

4.9.1. SCREEN: Software of the pressurized fluidic system displaying the reservoir providing fluid. *Videographer: Please film the screen as backup*

Results

5. Results: In Vivo Imaging of the Tongue Surface and Taste Screening

5.1. The tongue surface of a Pirt-GCaMP-tdTomato *(pert-G-camp-tee-dee-tomato)* mouse **[1]** is covered with auto fluorescent filiform papillae **[2]** and sparsely spread taste buds **[3]**.

5.1.1. LAB MEDIA: Figure 4A.

5.1.2. LAB MEDIA: Figure 4A. *Video Editor: Emphasize the yellow structures*

5.1.3. LAB MEDIA: Figure 4A. *Video Editor: Emphasize the taste bud in the center*

5.2. The filiform papillae, in yellow, are captured using a photodetector at 500 to 550 nanometer and can be observed from the very surface of the tongue up to approximately 25 micrometers in depth **[1]**.

5.2.1. LAB MEDIA: Figure 4. *Video Editor: Emphasize the bright-yellow structures in the filiform papillae panel in 4B.*

5.3. The GCaMP *(G-camp)* signals in green, detected by the 500 to 550-nanometer filter **[1]**, and the tdTomato *(tee-dee-tomato)* signals in red, detected by the 607 to 670-nanometer filter **[2]**, represent the taste cells **[3]**. The tdTomato signal is obtained for ratiometric analysis **[4]**.

5.3.1. LAB MEDIA: Figure 4. *Video Editor: Emphasize the green cells in the center of the GCaMP panel in 4B.*

5.3.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the red cells in the center of the tdTomato panel in 4B.*

5.3.3. LAB MEDIA: Figure 4. *Video Editor: Emphasize the green and red cells in the center of the GCaMP and tdTomato panels in 4B.*

5.3.4. LAB MEDIA: Figure 4. *Video Editor: Emphasize the tdTomato panel in 4B.*

5.4. The blood vessels that surround the taste bud are acquired using the 500 to 550-nanometer filter set **[1]** and the collagen connective tissue, which structurally supports the taste bud, is acquired using the 447 to 460-nanometer filter set **[2]**.

5.4.1. LAB MEDIA: Figure 4. *Video Editor: Emphasize the red Blood vessel in the blood vessel panel in 4B.*

5.4.2. LAB MEDIA: Figure 4. *Video Editor: Emphasize the blue Collagen in the collagen panel in 4B.*

5.5. In this representative example of in vivo taste screening using calcium imaging **[1]**, each taste cell is demarcated by dashed lines **[2]**. In this trial, cell 2 responded to both sweet **[3]** and umami tastants **[4]** and cell 3 responded to both low and high salt tastants **[5]**. None of the cells in this taste bud responded to sour tastes **[6]**.

5.5.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize figure 5A*

5.5.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize the cells marked by dashed lines in figure 5A.*

5.5.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize the sweet response for cell 2 in figure 5B.*

5.5.4. LAB MEDIA: Figure 5. *Video Editor: Emphasize the umami response for cell 2 in figure 5B.*

5.5.5. LAB MEDIA: Figure 5. *Video Editor: Emphasize both salty responses (salty H and salty L) for cell 3 in figure 5B*

5.5.6. LAB MEDIA: Figure 5. *Video Editor: Emphasize the lack of response in the sour column*

Conclusion

6. Conclusion Interview Statements

6.1. Since the purpose of this experiment is to observe taste cell function in the natural environment, it is important to deliver the fluorescence into the blood vessels at the preparation step **[1]** and confirm its circulation during the experiment **[2]**.

6.1.1. *3.1.2*

6.1.2. *4.6.1. 62361_screenshot_6. 0:15-0:21*