

Submission ID #: 68621

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=20929453>

Title: Translationally-Relevant Tumor Resection Model for Murine Preclinical Models of Oral Squamous Cell Carcinoma

Authors and Affiliations:

Gemalene M. Sunga^{1,2}, Alejandra Cobos Perez^{1,3}, Andrea H. Molina¹, Nourhan Hussein¹, Neeraja Dharmaraj¹, Andrew Sikora³, Simon Young¹

¹Katz Department of Oral and Maxillofacial Surgery, The University of Texas Health Science Center at Houston, School of Dentistry

²Department of Head and Neck Surgery, The University of Texas MD Anderson Cancer Center

³Department of Bioengineering, Rice University

Corresponding Authors:

Gemalene M. Sunga (Gemalene.Sunga@uth.tmc.edu)

Email Addresses for All Authors:

Gemalene M. Sunga (Gemalene.Sunga@uth.tmc.edu)

Alejandra Cobos Perez (ac256@rice.edu)

Andrea H. Molina (Andrea.H.Molina@uth.tmc.edu)

Nourhan Hussein (Nourhan.I.Hussein@uth.tmc.edu)

Neeraja Dharmaraj (Neeraja.Dharmaraj@uth.tmc.edu)

Andrew Sikora (AGSikora@mdanderson.org)

Simon Young (Simon.Young@uth.tmc.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? **No**

- 3. Filming location:** Will the filming need to take place in multiple locations? **Yes**
 - 6th floor animal housing facility
 - 5th floor office (BBSB 5336)
 - 4th floor lab

- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 27

Number of Shots: 55

Introduction

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**.

INTRODUCTION:

REQUIRED: Summarize your research focus and the main questions it aims to answer.

- 1.1. ~~Gemalene Sunga~~ **Andrea Molina:** This work focuses on the study of oral cancers, establishing a practical preclinical model for orthotopic tumor resection and recurrence.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Describe the research gap or limitation in existing methods and how this protocol addresses them.

- 1.2. ~~Gemalene Sunga~~ **Andrea Molina:** Surgery is a primary treatment modality for a majority of patients with oral cancer. This protocol allows for its investigation.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

Describe the most important consideration or challenge when performing this protocol.

- 1.3. ~~Gemalene Sunga~~ **Andrea Molina :** The most important consideration in using this protocol is consistency in the inoculation and establishment of tumors.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Are there any additional methods or analyses that can be performed following this procedure?

- 1.4. ~~Gemalene Sunga~~ **Andrea Molina :** Beyond this procedure, processing of resected tumors for analysis by flow cytometry or immunohistochemistry, for example, can be performed.

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

How can future studies build on this work? Describe the questions or research directions that can be investigated next.

- 1.5. **Gemalene Sunga Andrea Molina** : This can be combined with additional treatment, allowing for investigation of the tumor microenvironment in relation to resection and recurrence.

- 1.5.1. 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.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at The University of Texas Health Science Center at Houston. All animal cell lines, including murine oral cancer cells (MOC2), are approved for use on IBC protocol IBC-22-0051

Protocol

2. Orthotopic Buccal Mucosa Inoculation of MOC2 Oral Squamous Cell Carcinoma Cells in Mice

Demonstrators: Alejandra Cobos Perez, Gemalene Sunga

- 2.1. To begin, obtain culture flasks containing 80% confluent MOC2 (*Mock-Two*) oral cancer cells [1]. Aspirate the culture media from the flasks [2].
 - 2.1.1. WIDE: Talent holding a flask with MOC2 cells.
AUTHORS: Please note that JoVE requires the opening shot to be a wide, non-screen capture. This shot was added specifically to meet that requirement
 - 2.1.2. Talent aspirating and discarding the culture media from the flask.
- 2.2. Wash the attached cells with magnesium- and calcium-free PBS to remove remaining serum [1]. Add a sufficient volume to fully cover the surface of the vessel containing the cells, then aspirate [2].
 - 2.2.1. Talent adding phosphate-buffered saline to the culture flask.
 - 2.2.2. Shot of the flask showing the cell layer fully covered with buffer.
- 2.3. Now, add 0.05 percent trypsin to the culture flask to sufficiently cover the bottom surface [1]. Incubate the flask at 37 degrees Celsius with 5 percent carbon dioxide for approximately 5 minutes to allow cell detachment [2].
 - 2.3.1. Talent adding trypsin to the bottom of the culture flask.
 - 2.3.2. Talent placing the flask into the incubator.
- 2.4. When cell detachment is seen, quench the trypsin by adding at least 2 times the volume of complete media [1]. Mix gently to stop enzymatic activity [2].
 - 2.4.1. Talent adding complete media to the flask.
 - 2.4.2. Talent gently swirling the flask to mix.
- 2.5. Transfer the cell suspension to a centrifuge tube [1]. Then place the tube in the centrifuge set to 350 *g* at room temperature for 5 minutes [2]. Aspirate and discard the supernatant [3].
 - 2.5.1. Talent transferring the cell suspension into a centrifuge tube.
 - 2.5.2. Talent placing the tube into the centrifuge.
 - 2.5.3. Talent aspirating the supernatant after centrifugation.

- 2.6. Resuspend the cell pellet in cold PBS [1]. Remove an aliquot of the suspension for cell counting [2].
 - 2.6.1. Talent resuspending the pellet with cold phosphate-buffered saline.
 - 2.6.2. Talent removing a small aliquot for counting.
- 2.7. Using additional PBS, adjust the cell suspension to the optimized concentration for inoculation [1-TXT]. Keep the prepared cell suspension on ice until ready for use [2].
 - 2.7.1. Talent adjusting the cell concentration with phosphate-buffered saline. **TXT: Cell concentration: 3×10^4 MOC2 cells per 30 μ L volume**
 - 2.7.2. Talent placing the cell suspension on ice.
- 2.8. Mix the cell suspension thoroughly to ensure even cell distribution [1]. Collect the suspension into a 0.3-milliliter insulin syringe [2].
 - 2.8.1. Talent gently mixing the cell suspension tube.
 - 2.8.2. Talent drawing the suspension into an insulin syringe.
- 2.9. Hold the mouse with one hand, from the posterior neck so the anterior side faces the handler, and the head is angled upward [1]. Maintain the mouth in an open and accessible position [2].
 - 2.9.1. Talent positioning the sedated mouse.
 - 2.9.2. Shot showing the mouse positioned with the mouth accessible.
- 2.10. Carefully insert the syringe into the open mouth at a downward angle toward the left lower mandibular vestibule [1]. Advance the needle through the lower buccal mucosa [2].
 - 2.10.1. Shot of syringe insertion into the oral cavity.
 - 2.10.2. Shot showing needle placement through the buccal mucosa.
- 2.11. Deliver 30 microliters of the cell suspension into the target region [1]. Hold the syringe in place for 5 seconds [2], then slowly withdraw it to prevent tracking of cells [3].
 - 2.11.1. Talent injecting the cell suspension.
 - 2.11.2. Shot showing the syringe held in place.
 - 2.11.3. Talent slowly withdrawing the syringe.
- 2.12. Ensure the mouse returns to normal physical and behavioural condition following inoculation [1].
 - 2.12.1. Talent observing the mouse for normal movement and posture.
- 2.13. As tumors develop, monitor the mouse weight using a balance [1]. Assess tumor growth

with digital calipers [2-TXT].

2.13.1. Talent weighing the mouse on a balance.

2.13.2. Talent measuring tumor size with digital calipers. **TXT: Monitor anesthetized mouse for tumor development 2x per week**

3. Orthotopic Tumor Resection in a Mouse Oral Cancer Model

Demonstrator: Gemalene Sunga

3.1. Select a clean procedure room with a closable door and limited traffic [1]. Place a new cage containing clean, dry bedding and a paper towel nest partially on an electric warmer to pre-warm it [2].

3.1.1. WIDE: Empty procedure room with door closed.

3.1.2. Talent positioning a clean cage on an electric warmer.

3.2. Warm a bead sterilizer to an operating temperature of 250 to 265 degrees Celsius [1].

3.2.1. Shot of bead sterilizer being heated.

3.3. Turn on a water-heated surgical pad and set it to 38.9 degrees Celsius [1-TXT].

3.3.1. Talent adjusting the surgical pad temperature. **TXT: Sanitize work surface with disinfectant**

Author's NOTE: Move step 3.4 after step 3.10.

3.4. Wearing sterile gloves and appropriate personal protective equipment, prepare a sterile field of at least 18 inches wide by 26 inches long [1]. Lay out all required sterile tools and materials [2].

3.4.1. Talent donning sterile gloves and protective equipment. **Videographer's NOTE: 3.4.1 & 3.4.2 - Same Shot, together**

3.4.2. Overhead shot of sterile instruments arranged on the field.

3.5. Apply a small amount of hair removal cream to the tumor resection site on an anesthetized mouse using a cotton-tip applicator [1]. Ensure sufficient margins to keep hair out of the incision area [2]. After 10 seconds, wipe away hair using dry, clean gauze [3].

3.5.1. Talent applying hair removal cream to the site. **Videographer's NOTE: Added shaving the mouse as well, but do not use it if not required. Use applying the hair removal cream as priority**

3.5.2. Shot of the treated area. **Videographer's NOTE: 3.5.1 - 3.6.to – shot together**

- 3.5.3. Talent wiping away hair with gauze.
- 3.6. Remove any residual cream using gauze dampened with saline or water [1]. Then dry the area with clean, dry gauze [2].
 - 3.6.1. Talent cleaning the site with damp gauze.
 - 3.6.2. Talent drying the site with clean, dry gauze.
- 3.7. Place the anesthetized mouse on the heating pad in a supine position with the anterior side facing upward [1-TXT]. Position the nose just inside the nose cone [2].
 - 3.7.1. Shot of the Mouse positioned on the heating pad. **TXT: Anesthesia: 1 - 3% Isoflurane**
 - 3.7.2. Shot of proper nose cone placement.
- 3.8. Now apply a sufficient amount of sterile lubricant eye ointment to each eye to prevent drying during the procedure [1].
 - 3.8.1. Shot showing both eyes protected.
- ~~3.9. After wearing appropriate personal protective equipment, prepare a sterile field of at least 18 inches wide by 26 inches long [1]. Lay out all required sterile tools and materials [2].~~
 - ~~3.9.1. Talent donning sterile gloves and protective equipment.~~
 - ~~3.9.2. Shot of sterile instruments arranged on the field.~~
- Author's NOTE: Redundant. Delete step 3.9.**
- 3.10. Using a clean cotton-tip applicator, swab the resection site with 70 percent isopropyl alcohol [1] followed by 10 percent iodine [2-TXT]. Remove the iodine after the last pass with 70% isopropyl alcohol [3].
 - 3.10.1. Talent swabbing with isopropyl alcohol.
 - 3.10.2. Talent applying iodine solution. **TXT: Repeat alternate washes of alcohol and iodine 3x**
 - 3.10.3. Talent removing iodine with alcohol.
- Author's NOTE: Insert step 3.4 here.**
- 3.11. Cover the mouse with a sterile drape [1]. Using sterile scissors, cut an access hole above the resection site [2].
 - 3.11.1. Talent placing a sterile drape over the mouse.
 - 3.11.2. Talent cutting the access hole using scissors.
- 3.12. With a new surgical scalpel, create a submandibular incision just below the tumor along the left jawline [1]. Using Hartman hemostats, gently widen the incision opening toward the tumor [2]. Dab any blood with sterile gauze as needed [3].

- 3.12.1. Talent using a scalpel to make a submandibular incision just below the tumor along the left jawline.
- 3.12.2. Talent widening the incision with hemostats.
- 3.12.3. Talent blotting blood with gauze.
- 3.13. Locate the tumor and hold it in place using forceps [1]. Using surgical scissors, snip around the base of the tumor to resect all visible tissue [2].
 - 3.13.1. Shot of forceps stabilizing the tumor.
 - 3.13.2. Talent cutting the base of the tumor to resect all visible tissue.
- 3.14. Reposition the skin over the resected tumor site [1]. Then close the incision with 4 to 6 interrupted sutures using 4-0 (*Four-Oh*) long-lasting vicryl sutures [2].
 - 3.14.1. Talent repositioning the skin over the site.
 - 3.14.2. Talent suturing the incision.
- 3.15. After closing the incision, gently transfer the mouse to the pre-warmed cage [1]. Monitor the mouse for return of consciousness and mobility until full recovery [2]. Immediately provide softened food by placing water-soaked pellets into an accessible container in the cage [3-TXT].
 - 3.15.1. Talent placing the mouse into the warm cage.
 - 3.15.2. Shot of mouse moving normally inside the cage.
 - 3.15.3. Talent placing softened food into the cage. **TXT: Allow mouse to resume normal solid food if no complications occur**

Results

4. Results

- 4.1. Using the MOC2 cell line, tumors were established in the murine buccal mucosa of the oral cavity [1].
 - 4.1.1. LAB MEDIA: Figure 2A–E. *Video editor: Sequentially show the images from A to E*
- 4.2. Tumor growth kinetics were characterized prior to resection [1].
 - 4.2.1. LAB MEDIA: Figure 3A.
- 4.3. Following tumor resection, there was an observed delay in tumor growth in the days immediately after surgery compared with unresected tumors [1], as shown by reduced tumor area shortly after resection [2].
 - 4.3.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the red line representing resected tumors*
 - 4.3.2. LAB MEDIA: Figure 3B. *Video editor: Highlight the portion of the graph where the resected group remains at or near zero tumor area while the unresected group increases.*
- 4.4. A minor extension in overall survival was observed in the resected group compared with the unresected control group, although this difference was not statistically significant [1].
 - 4.4.1. LAB MEDIA: Figure 3C.
- 4.5. Unresected tumors reached euthanasia criteria by 12 days post-surgery [1], whereas the resected group first began to lose mice at day 19 [2].
 - 4.5.1. LAB MEDIA: Figure 3C. *Video editor: Highlight the point on the black unresected survival curve where it begins to drop around day 12.*
 - 4.5.2. LAB MEDIA: Figure 3C. *Video editor: Highlight the point on the red resected survival curve where it first drops around day 19.*

1. Confluent

Pronunciation link: <https://www.merriam-webster.com/dictionary/confluent>

IPA: /kən'flu:ənt/

Phonetic Spelling: kuhn·floo·uhnt

2. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æs.pəˌreɪt/
Phonetic Spelling: as·puh·rayt
3. Magnesium
Pronunciation link: <https://www.merriam-webster.com/dictionary/magnesium>
IPA: /mæg'niː.zi.əm/
Phonetic Spelling: mag·nee·zee·uhm
4. Phosphate-buffered
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /'fɑːs.fert/
Phonetic Spelling: faahs·fayt
5. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪp.sɪn/
Phonetic Spelling: trip·sin
6. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪŋ.kjəˌbeɪt/
Phonetic Spelling: ing·kyuh·bayt
7. Detachment
Pronunciation link: <https://www.merriam-webster.com/dictionary/detachment>
IPA: /dɪ'tætʃ.mənt/
Phonetic Spelling: dih·tach·muhnt
8. Enzymatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>
IPA: /ˌɛn.zə'mæt.ɪk/
Phonetic Spelling: en·zy·mat·ik
9. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sɛn.trəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
10. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuː.pərˈneɪ.tənt/
Phonetic Spelling: soo·per·nay·tuht
11. Resuspend
Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
IPA: /ˌriː.sə'spɛnd/
Phonetic Spelling: ree·suh·spend
12. Aliquot
Pronunciation link: <https://www.merriam-webster.com/dictionary/aliquot>
IPA: /'æl.ɪ.kwɑːt/
Phonetic Spelling: al·ih·kwaat

13. Inoculation
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculation>
IPA: /ɪˌnɑː.kjəˈleɪ.ʃən/
Phonetic Spelling: ih·naak·yuh·lay·shuhn
14. Mandibular
Pronunciation link: <https://www.merriam-webster.com/dictionary/mandibular>
IPA: /mænˈdɪb.jə.lər/
Phonetic Spelling: man·dib·yuh·ler
15. Vestibule
Pronunciation link: <https://www.merriam-webster.com/dictionary/vestibule>
IPA: /ˈvɛs.təˌbjʊ:l/
Phonetic Spelling: ves·tuh·byool
16. Buccal
Pronunciation link: <https://www.merriam-webster.com/dictionary/buccal>
IPA: /ˈbʌk.əl/
Phonetic Spelling: buhk·uhl
17. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪ.kroʊˌliː.tər/
Phonetic Spelling: my·kroh·lee·ter
18. Behavioral
Pronunciation link: <https://www.merriam-webster.com/dictionary/behavioral>
IPA: /bɪˈheɪ.vjə.əl/
Phonetic Spelling: bih·hayv·yuh·ruhl
19. Orthotopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/orthotopic>
IPA: /ˌɔːr.θəˈtɑː.pɪk/
Phonetic Spelling: or·thuh·taa·pik
20. Sterilizer
Pronunciation link: <https://www.merriam-webster.com/dictionary/sterilizer>
IPA: /ˈster.əˌlaɪ.zər/
Phonetic Spelling: ster·uh·ly·zer
21. Isopropyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropyl>
IPA: /ˌaɪ.souˈproʊ.pəl/
Phonetic Spelling: eye·soh·proh·puhl
22. Submandibular
Pronunciation link: <https://www.merriam-webster.com/dictionary/submandibular>
IPA: /ˌsʌb.mænˈdɪb.jə.lər/
Phonetic Spelling: sub·man·dib·yuh·ler
23. Hemostats
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostat>
IPA: /ˈhiː.moʊˌstæt/
Phonetic Spelling: hee·moh·stat

24. Forceps
Pronunciation link: <https://www.merriam-webster.com/dictionary/forceps>
IPA: /'fɔːr.sɛps/
Phonetic Spelling: for·seps
25. Sutures
Pronunciation link: <https://www.merriam-webster.com/dictionary/suture>
IPA: /'suː.tʃər/
Phonetic Spelling: soo·cher
26. Vicryl
Pronunciation link: <https://www.howtopronounce.com/vicryl>
IPA: /'vaɪ.krɪl/
Phonetic Spelling: vy·kril
27. Murine
Pronunciation link: <https://www.merriam-webster.com/dictionary/murine>
IPA: /'mjɔr.ən/
Phonetic Spelling: myoor·ine
28. Kinetics
Pronunciation link: <https://www.merriam-webster.com/dictionary/kinetics>
IPA: /kə'net.ɪks/
Phonetic Spelling: kuh·net·iks
29. Resection
Pronunciation link: <https://www.merriam-webster.com/dictionary/resection>
IPA: /rɪ'sek.ʃən/
Phonetic Spelling: ree·sek·shuhn
30. Statistically
Pronunciation link: <https://www.merriam-webster.com/dictionary/statistically>
IPA: /stə'tɪs.tɪ.kli/
Phonetic Spelling: stuh·tis·ti·klee
31. Euthanasia
Pronunciation link: <https://www.merriam-webster.com/dictionary/euthanasia>
IPA: /juː.θə'nei.zə/
Phonetic Spelling: yoo·thuh·nay·zhuh