FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 67848
Scriptwriter Name: Sulakshana Karkala
Project Page Link: https://review.jove.com/account/file-uploader?src=20693688

Title: Murine Model of Advanced Periodontitis Induced by Nylon Ligature in the Second Upper Molar

Authors and Affiliations:
Betsaida J. Ortiz-Sánchez[1,2], Imelda Juárez-Avelar[2,3], Miriam Rodriguez-Sosa[2]

[1]Undergraduate Program of Dental Surgeon, Facultad de Estudios Superiores Iztacala (FES-Iztacala), Universidad Nacional Autónoma de Mexico (UNAM)
[2]Innate Immunity Laboratory, Biomedicine Research Unit (UBIMED), FES-Iztacala, UNAM
[3]Doctoral Program in Biological Sciences, UNAM

Corresponding Authors:
Miriam Rodriguez-Sosa (rodriguezm@unam.mx)
Email Addresses for All Authors:
Betsaida J. Ortiz-Sánchez (b.ortiz@unam.mx)
Imelda Juárez-Avelar (imelda.juarez@iztacala.unam.mx)
Miriam Rodriguez-Sosa (rodriguezm@unam.mx)

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? **Yes, done**

   SCOPE: 2.4.1-2.4.2, 2.5.1, 2.6.1-2.6.3,2.7.1-2.7.3, 2.8.1-2.8.4

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, 500 m apart.**

Current Protocol Length
Number of Steps: 19
Number of Shots: 46

Introduction

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

1.1. **Betsaida J. Ortiz-Sánchez:** The scope of our research is to study the cellular and molecular factors that favor periodontitis, for which we use a murine model of ligation-induced periodontitis.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:3.1*

What are the most recent developments in your field of research?

1.2. **Miriam Rodriguez Sosa: ~~Betsaida J. Ortiz-Sánchez~~:** Recent research has established the importance of inflammation, dysbiosis, and systemic diseases as determining factors in the development of periodontitis.

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

What technologies are currently used to advance research in your field?

1.3. **Betsaida J. Ortiz-Sánchez:** The technology recently used in periodontics includes animal models, some knockout for different molecules, proteomics, sequencing, flow cytometry and histology, among others.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.2*

What are the current experimental challenges?

1.4. **Miriam Rodriguez-Sosa:** Current experimental challenges are related to understanding the interaction between systemic diseases and periodontitis.

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

What significant findings have you established in your field?

1.5. **Imelda Juarez Avelar: ~~Betsaida J. Ortiz-Sánchez~~:** We used a murine model of ligature periodontitis to demonstrate that the proinflammatory cytokine MIF is involved in the development of periodontitis during pregnancy.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:2.8*

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

Testimonial Questions (OPTIONAL):

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Betsaida J. Ortiz-Sánchez:** We believe publishing an improved periodontitis protocol in JoVE will be useful to other researchers, making us more visible, and have a positively impacting ~~their understanding of our~~ in research.

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.7. **Miriam Rodriguez-Sosa:** We hope that other researchers in the field will learn about our work. Identifying us as a formal working group in the field of periodontology will open opportunities for collaboration to improve our laboratory's productivity.

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

AUTHORS: Please present the testimonials in both Spanish and English
Videographer: Please capture the testimonials in both Spanish and English

Ethics Title Card

This research has been approved by the Ethics Committee at Facultad de Estudios Superiores Iztacala CE/FESI/072024/1765

Protocol

2. Ligature-Induced Periodontal Disease in Mice

Demonstrators: Betsaida Ortiz, Yunuen Mendoza, Diego Patlani, Brandon Zamora

2.1. To begin, place an anesthetized mouse on a worktable facing upward with its head directed toward the operator **[1-TXT]**. Gently pull each leg without tension **[2]** and secure them with micropore tape to prevent sudden involuntary movements **[3]**. Cover the mouse with a blanket or gauze to maintain its body heat **[4]**.

2.1.1. WIDE: Talent positioning the mouse on the worktable. **TXT: Anesthesia: Xylazine (1 mg/kg) and ketamine (2 mg/kg) injection**

2.1.2. Shot of the leg being pulled gently. **Videographer's NOTE:** Shot slated as 2.1.2 contains also: 2.1.3 , 2.1.4 , 2.2.1 , 2.2.2 , 2.3.1 and 2.3.2 ; all indicated via audio except 2.3.2 which took place at time: 2:05

2.1.3. Talent taping each leg of the mouse.

2.1.4. Talent covering the mouse with gauze.

2.2. Place one end of an orthodontic elastic around the upper incisors and secure the other end to an upper holder without applying tension **[1]**. Then, place a second orthodontic elastic around the inferior incisors and secure it to an inferior holder using a rubber band **[2]**.

2.2.1. Talent attaching the elastic to the upper incisors and securing to the holder. **Videographer's NOTE:** Shots 2.2.1 and 2.2.2 were mentioned in a swap order, by my mistake.

2.2.2. Talent repeating the process for the lower incisors.

2.3. Now insert the cheek separators **[1]** and carefully move the tongue to one side to improve visibility **[2]**.

2.3.1. Talent inserting cheek separators

2.3.2. Shot of the tongue being moved to the other side. **Videographer's NOTE:** 2.3.2 took place at time: 2:05 in the shot slated 2.1.2

2.4. Position the microscope to visualize the upper molars using the lowest magnification objective **[1]**. Once located, increase magnification to optimize comfort and focus for the operator **[2]**.

2.4.1. SCOPE: 2.4.1-scope-lowest-magnif.mpg.

2.4.2. SCOPE: 2.4.2-scope-higher-magnification-ok.mp4.

2.5. When the image is clear, identify the largest and proximal molar M1, the next molar M2, and the smallest and distal molar M3 **[1]**. Use a 6-0 *(six-zero)* nylon suture for

ligature placement as it causes less mechanical damage compared to wider sutures **[2]**.

2.5.1. SCOPE: 2.5.1-Scope-View-M1-M2-M3.mp4.
AUTHORS: Please point to M1, M2 and M3 in sequence

2.5.2. Talent holding 6-0 nylon suture and preparing for ligature. **Videographer's NOTE:** Shot 2.5.2 includes a camera movement because the talent was rushing a bit because of the time of the anesthesia was running out. The 2nd placement of the camera did not succeed in get a sharp focus nor a clear view of the nylon suture.

2.6. Using tweezers, remove the distal tip of the 6-0 nylon suture **[1]** and place it at the base of the papilla from the palatine side at the distal of M2 **[2]**. Apply light pressure through the base of the interproximal palatal space to advance it toward the buccal surface **[3]**.

2.6.1. SCOPE: 2.6.1-scope-tip-in-distal_2.mp4.
2.6.2. SCOPE: 2.6.2-scope-nylon.mpg.
2.6.3. SCOPE: 2.6.3-scope-pressure-towards-palatine.mp4.

2.7. When the suture crosses, pull it through the interproximal space toward the buccal side **[1]**. Now, place the tip of the suture at the base of the interproximal area at the mesial surface of M2 from the buccal side **[2]**. Push it gently to cross the interproximal space and pass it back through the buccal space to the palatine **[3]**.

2.7.1. SCOPE: 2.7.1-scope-suture-pulled.mp4.
2.7.2. SCOPE: 2.7.2-scope-suture-place-in-buccal.mp4.
2.7.3. SCOPE: 2.7.3-scope-suture-pushed.mp4.

2.8. Gently pull on the 6-0 nylon suture **[1]**, hold the tip with tweezers, adjust it around M2 **[2]**, and secure the suture using three simple knots **[3]**. Then cut the suture with fine scissors **[4]**.

2.8.1. SCOPE: 2.8.1-scope-suture-pulled.mp4.
2.8.2. SCOPE: 2.8.2-scope-sutures-adjusted.mp4.
2.8.3. SCOPE: 2.8.3-scope-sutures-secured.mp4.
2.8.4. SCOPE: 2.8.4-scope-sutures-cut.mp4.

2.9. To release the mouse, remove the cheek separators and micropore tape from the legs **[1]**. Then, remove the elastic around the lower incisors, and the upper incisors **[2]**.

2.9.1. Talent removing all securing accessories. **Videographer's NOTE:**Shot slated 2.9.1 contains also 2.9.2 , 2.10.1 , 2.10.2 and 2.10.3..

2.9.2. Talent releasing elastics from lower and upper incisors.

2.10. Now remove the mouse from the worktable **[1]** and keep the tongue to one side to maintain an open airway and prevent blockage **[2].** Wrap the mouse in gauze or cloth, and place it face up **[3]**.

2.10.1. Talent removing the mouse from the worktable.

2.10.3 Talent adjusting tongue to ensure open airway. **NOTE**: 2.10.3 comes before 2.10.2. VO adjusted

Videographer's NOTE:2.10.3 took place at time: 1:33, but it is not clearly visible

2.10.2 Talent wrapping mouse and positioning it safely.

2.11. Keep the animal warm, covered with cloth or gauze, and under observation until it is completely awake **[1]**. Then, return it to its regular cage **[2-TXT]**. Apply one drop of hypromellose in each eye until the mouse can blink normally **[3]**.
2.11.1. Talent monitoring mouse recovery.
2.11.2. Talent placing mouse back in cage. **TXT: Keep animal under standard conditions Videographer's NOTE:** order of Shot 2.11.2 and 2.11.3 were swapped during filming
2.11.3. Talent applying hypromellose to each eye.

2.12. To check the permanence of the ligature, take the mouse **[1-TXT]**, hold its head and body firmly, and use tweezers to open the snout **[2]**. Under lamp light, observe the 6-0 nylon suture around M2 **[3-TXT]**.
2.12.1. Talent holding mouse. **TXT: Check permanence weekly**
2.12.2. Talent opening snout with tweezers
2.12.3. LAB MEDIA: 2.13.1-suture-revision.mp4. **TXT: Observe biofilm and tissue irritation to assess periodontal disease**

~~2.13. Determine the development of periodontal disease based on visible biofilm accumulation and tissue irritation **[1]**.~~
~~2.13.1. Shot of the biofilm around the teeth.~~
~~**AUTHORS:** For this shot, please point to the biofilm~~
~~SCOPE: Mouse under stereoscopic microscope that shows the ligature in position, the biofilm can not be point at, it is too small~~

2.14. For the confirmation of biofilm formation using histology, harvest the maxillae from euthanized animals after 30 days **[1-TXT]**. Wash the tissues in 0.9% sodium chloride solution **[2]** and place them into labeled microcentrifuge tubes **[3]**.
2.14.1. Shot of harvested maxillae. **TXT: Euthanasia: CO_2 inhalation**
2.14.2. Talent placing the samples in 0.9% sodium chloride.
2.14.3. Talent transferring washed maxillae into labeled tubes.

2.15. Fix the samples in 4% paraformaldehyde solution for 2 hours under agitation **[1]**. Then wash the fixed samples with tap water for 2 hours **[2]**.
2.15.1. Talent placing samples in paraformaldehyde solution on agitator.
2.15.2. Talent transferring fixed samples to labware containing tap water.

2.16. Next, decalcify the samples in twenty volumes of 4% EDTA *(E-D-T-A)* at pH 7.3 for 20 days, changing the EDTA every 4 days **[1]**. Then embed the samples in paraffin **[2]**.
2.16.1. Talent transferring the samples into labeled vials containing 20 volumes of 4% EDTA.
2.16.2. Shot of samples embedded in paraffin.

2.17. Cut 5-micrometer sections from the M2 area **[1]** and stain them with Hematoxylin and Eosin **[2]**.
2.17.1. Shot of 5 µm sliced sections of M2.
2.17.2. Talent adding H&E stain over the sections. **Videographer's NOTE**: Clip "EEC_2580" is 2.17.1-2 (Take 2) and is a shot suggested by the authors as an extra shot
AND
Shot slated 2.17.3 should have been slated as 2.17.1-3 (Take 3) and is a shot suggested by the authors as an extra shot.

2.18. Observe the stained sections under an optical microscope to describe changes in sulcus epithelium, gingival and periodontal fibers, height, and alveolar crest integrity **[1]**. Measure attachment loss by determining the distance between the cementoenamel junction and highest point of the bone crest using a digital camera **[2]**.
2.18.1. SCOPE: 2.18.1.mp4 00:00-00:07
Video Editor: Please slow down the video or pause frame
2.18.2. SCOPE: 2.18.2.mp4 00:05-00:35

2.19. After histometric analysis, analyze data from control and periodontal treatment groups using the Mann–Whitney U test **[1]**.
2.19.1. Talent looking at statistics data on the computer. **TXT: Consider p-value < 0.05 for significance**

Results

3. Representative Results

3.1. Periodontitis-induced tissue in mice showed inflammation, bleeding and periodontal pocket formation at the gingival margin by day 30, in contrast to the control group **[1]**.

3.1.1. LAB MEDIA: Figure 5. *Video editor: First, show the leftmost image labeled "CTL" with its clean white ridges, then focus on the middle image labeled "Day 1" with the blue suture, and finally highlight the rightmost image labeled "Day 30" showing the pink, swollen area with exposed ridges.*

3.2. Histological analysis confirmed significant structural damage in the PT *(P-T)* group, with presence of periodontal pockets **[1-TXT]**, detachment of fibers and apical migration of the epithelium **[2]**. Loss of cell nuclei in gingiva and bone of the buccal and palatal sides were also seen **[3]**.

3.2.1. LAB MEDIA: Figure 6A PT *Video Editor: please highlight area pointed at by green arrow* **TXT: PT: Periodontitis**

3.2.2. LAB MEDIA: Figure 6A PT *Video Editor: please highlight area pointed at by orange arrow*

3.2.3. LAB MEDIA: Figure 6A PT *Video Editor: please highlight area pointed at by Blue arrow*

3.3. In contrast, the CTL *(C-T-L)* group showed healthy histology **[1-TXT]**.

3.3.1. LAB MEDIA: Figure 6A CTL . *Video editor: Please highlight the areas pointed at by the blue, green and orange arrows* **TXT: CTL: Control**

3.4. Quantitative analysis revealed significantly increased attachment loss in the PT group with buccal side loss at approximately 209 micrometers **[1]**, and palatine side at approximately 254 micrometers **[2]**.

3.4.1. LAB MEDIA: Figure 6B *Video editor: Please highlight the purple column labelled PT*

3.4.2. LAB MEDIA: Figure 6C *Video editor: Please highlight the purple column labelled PT*

Pronunciation Guide

1. **Xylazine**
   Pronunciation link: https://www.merriam-webster.com/dictionary/xylazine
   IPA: /ˈzaɪ.ləˌziːn/
   Phonetic Spelling: zy-luh-zeen
2. **Ketamine**
   Pronunciation link: https://www.merriam-webster.com/dictionary/ketamine
   IPA: /ˈkɛtəˌmiːn/
   Phonetic Spelling: keh-tuh-meen
3. **Micropore**
   No confirmed link found
   IPA: /ˈmaɪ.krəˌpɔːr/
   Phonetic Spelling: my-kruh-pohr
4. **Incisors**
   Pronunciation link: https://www.merriam-webster.com/dictionary/incisor
   IPA: /ɪnˈsaɪ.zɚz/
   Phonetic Spelling: in-sy-zurz
5. **Buccal**
   Pronunciation link: https://www.merriam-webster.com/dictionary/buccal
   IPA: /ˈbʌk.əl/ or /ˈbuː.kəl/ (preferred: /ˈbʌk.əl/)
   Phonetic Spelling: buk-uhl
6. **Papilla**
   Pronunciation link: https://www.merriam-webster.com/dictionary/papilla
   IPA: /pəˈpɪl.ə/
   Phonetic Spelling: puh-pil-uh
7. **Interproximal**
   No confirmed link found
   IPA: /ˌɪn.tɚˈprɑk.sə.məl/
   Phonetic Spelling: in-ter-prok-suh-muhl
8. **Ligature**
   Pronunciation link: https://www.merriam-webster.com/dictionary/ligature
   IPA: /ˈlɪg.ə.tʃɚ/
   Phonetic Spelling: lig-uh-chur
9. **Hypromellose**
   Pronunciation link: https://www.howtopronounce.com/hypromellose
   IPA: /ˌhaɪ.proʊˈmɛl.oʊs/
   Phonetic Spelling: hy-proh-mel-ohs
10. **Maxillae**
    Pronunciation link: https://www.merriam-webster.com/dictionary/maxilla
    IPA: /ˈmæk.sɪ.li/
    Phonetic Spelling: mak-sih-lee

11. **Paraformaldehyde**
    Pronunciation link: https://www.merriam-webster.com/dictionary/paraformaldehyde
    IPA: /ˌpær.əˈfɔr.məlˌdaɪd/
    Phonetic Spelling: par-uh-for-muhl-dyde
12. **EDTA**
    Pronunciation link: https://www.howtopronounce.com/edta
    IPA: /ˌiː.diː.tiːˈeɪ/
    Phonetic Spelling: ee-dee-tee-ay
13. **Hematoxylin**
    Pronunciation link: https://www.merriam-webster.com/dictionary/hematoxylin
    IPA: /ˌhiː.məˈtɑːk.sə.lɪn/
    Phonetic Spelling: hee-muh-tok-suh-lin
14. **Eosin**
    Pronunciation link: https://www.merriam-webster.com/dictionary/eosin
    IPA: /ˈiː.ə.sɪn/
    Phonetic Spelling: ee-uh-sin
15. **Cementoenamel**
    No confirmed link found
    IPA: /sɪˌmɛn.toʊ.ɪˈnæ.məl/
    Phonetic Spelling: si-men-toh-ih-na-muhl
16. **Histometric**
    No confirmed link found
    IPA: /ˌhɪs.toʊˈmɛ.trɪk/
    Phonetic Spelling: his-toh-meh-trik
17. **Periodontal**
    Pronunciation link: https://www.merriam-webster.com/dictionary/periodontal
    IPA: /ˌpɛr.i.oʊˈdɑːn.təl/
    Phonetic Spelling: peh-ree-oh-don-tuhl
18. **Gingival**
    Pronunciation link: https://www.merriam-webster.com/dictionary/gingival
    IPA: /ˈdʒɪn.dʒə.vəl/
    Phonetic Spelling: jin-juh-vuhl