

Submission ID #: 69895

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=21318023>

Title: Design and Standardization of a Mandibular Injury Model for the Study of Craniofacial Tissue Regeneration in *the Ambystoma mexicanum* Model

Authors and Affiliations:

Samanta Tarquino González^{1,2*}, Belfran Carbonell Medina^{1,2,3*}

¹Departamento de Estudios Básicos Integrados, Facultad de Odontología, Universidad de Antioquia

²Grupo Genética, Regeneración y Cáncer, Facultad de Ciencias Exactas y Naturales

³Instituto de Biología, Universidad de Antioquia

***These authors contributed equally**

Corresponding Authors:

Belfran Carbonell Medina (belfran.carbonell@udea.edu.co)

Email Addresses for All Authors:

Samanta Tarquino González (Samanta.tarquino@udea.edu.co)

Belfran Carbonell Medina (belfran.carbonell@udea.edu.co)

Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

☒ Correct

☐ Incorrect

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

Authors: Please create scope videos of the shots labeled as SCOPE and upload the files to your project page as soon as possible: <https://review.jove.com/account/file-uploader?src=21318023>

SCOPE: 2.7.2 , 2.11.1-2.11.2, 2.12.1-2.12.2

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: 02/07/2026

When you are ready to submit your video files, please contact our Content Manager, [Utkarsh Khare](#)

Current Protocol Length

Number of Steps: 12

Number of Shots: 29

Introduction

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Belfran Medina:** The scope of this research is to understand how craniofacial tissues regenerate morphologically, structurally, and functionally using an axolotl jaw amputation model.

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

~~What are the current experimental challenges?~~

- 1.2. **Samanta González:** The current experimental challenges involve developing practical, safe, and reproducible surgical axolotl models to reliably investigate and compare craniofacial regeneration mechanisms.

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

CONCLUSION:

~~What advantage does your protocol offer compared to other techniques?~~

- 1.3. **Samanta González:** The advantage of this protocol is that it enables controlled injuries, is easy to perform, preserves feeding ability, and maintains animal viability.

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

~~How will your findings advance research in your field?~~

- 1.4. **Samanta González:** Our findings will advance research in the field by enabling systematic comparisons of surgical designs across axolotl and other species with effective craniofacial regeneration.

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

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

- 1.5. **Samanta González:** These results motivate the identification and elucidation of signaling pathways regulating the cellular and molecular mechanisms underlying this regenerative response.

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

Ethics Title Card

This research has been approved by the Animal Experimentation Ethics Committee of the University of Antioquia

Protocol

2. Complete Transverse Amputation in the Distal Third of the Axolotl Mandible

~~Distal Mandible Amputation and Regenerative Imaging in Axolotls~~

Demonstrators: Samanta Tarquino González and Belfran Carbonell Medina

- 2.1. To begin, select an axolotl approximately 12 to 15 centimeters in length from snout to tail [1]. Place the selected axolotl in a transparent rectangular container filled with 40 percent Holtfreter's solution [2]. Maintain the solution temperature between 19 to 21 degrees Celsius and keep the animal under a 12 to 12 light-dark cycle. Avoid overfeeding the animal 24 hours prior to experimentation [3]. **NOTE: VO altered**
 - 2.1.1. Talent selecting axolotl and examining body length with hands.
 - 2.1.2. Talent placing a single axolotl into a transparent rectangular container.
 - 2.1.3. Talent showing the filled container with animal and measuring temperature of the bath.
- 2.2. To perform complete distal mandible amputation, first carefully place a deeply anesthetized axolotl supine on the plastic tray [1-TXT]. Measure the amputation region with a calliper on the outer edge of the jaw toward the proximal end at approximately 2.5 millimeters in the chin and symphysis region [2].
 - 2.2.1. Talent placing anesthetized axolotl on plastic tray. **TXT: Anesthesia: Submergence in 0.1% Tricaine methanesulfonate anesthetic solution (20 – 30 min)**
 - 2.2.2. Talent using a calliper to measure jaw region.
- 2.3. Make a mark with a red wax pencil to identify the trajectory of the amputation plane and the extent of the surgical defect [1]. Then carefully lift the axolotl and hold it firmly with one hand [2].
 - 2.3.1. Talent drawing a red wax line marking the cut plane.
 - 2.3.2. Talent lifting and holding the axolotl securely.
- 2.4. Position a direct light over the head region of the axolotl, specifically over the anatomical region to be operated on [1]. With the other hand, use the rounded, wider side of a 7A spatula to separate the tongue from the floor of the mouth and the jaw region to be operated on [2].

- 2.4.1. Talent positions a surgical light over operation site.
- 2.4.2. Shot of 7A spatula being used to lift the axolotl tongue.
- 2.5. Now, make an initial cut with surgical forceps from the outer edge of the jaw through skin and hard tissues including tooth bone and Meckel's cartilage [1]. Then use fine surgical scissors to cut transversely across the entire width of the jaw following the planned plane [2]. Observe the immediate retraction of remaining soft tissue [3].
 - 2.5.1. Shot of an initial cut being made with forceps from the outer edge of the jaw through skin and hard tissues
 - 2.5.2. Shot of scissors cutting transversely across entire width of the jaw.
 - 2.5.3. Shot of soft tissue retracting post-cut.
- 2.6. Collect the amputated tissue with dissecting forceps [1] and immerse it in a 1.5-milliliter tube containing 4 percent paraformaldehyde for fixation or Trizol for nucleic acid extraction [2].
 - 2.6.1. Talent collecting tissue with forceps.
 - 2.6.2. Shot of tissue placed into labeled tube with solution.
- 2.7. Transfer the axolotl into a Petri dish [1] and observe under the stereomicroscope to remodel exposed bone and cartilage edges [2]. Then carefully return the axolotl to the plastic tray [3] and apply 0.5 percent Sulfamerazine antibiotic baths for 12 hours [4].
 - 2.7.1. Talent placing the axolotl into a Petridish.
 - 2.7.2. SCOPE: 2.7.2.-SCOPE-avi 00:06-00:10 *when saying observe under scope* and 00:33-00:40 *when saying remodel exposed bone.....*
 - 2.7.3. Talent placing axolotl back onto tray .
 - 2.7.4. Talent placing tray in 0.5 percent Sulfamerazine antibiotic solution.

Postoperative Recovery and Monitoring of Regenerative Response

NOTE: Treat this as a separate section and add the above title card

- 2.8. After surgery, place the axolotl in a transparent container with cold 40 percent Holtfreter's solution at approximately 4 to 8 degrees Celsius [1]. Observe for return of gill movements, righting reflex, and tactile response over 30 to 60 minutes as signs of recovery [2].
 - 2.8.1. Talent placing recovering axolotl into chilled solution.
 - 2.8.2. Shot of gills and righting reflex behavior.

- 2.9. Change the cold Holtfreter solution every 24 hours for at least 8 days, paying special attention to the first 48 hours [1]. After 24 to 48 hours post-procedure, feed the axolotl soft-textured food that is easier to catch and less traumatic [2].
- 2.9.1. Talent changing Holtfreter's solution.
- 2.9.2. Talent offering soft food to axolotl.
- 2.10. For image acquisition, transfer the anesthetized axolotl into a Petri dish under the stereomicroscope with a digital camera [1-TXT]. Use incident light pointing at the head to improve visibility of the jaw area [2] and hold the body securely to prevent slipping [3-TXT].
- 2.10.1. Talent transferring anesthetized axolotl to imaging station. **TXT: Anesthesia: Submergence in 0.1% Tricaine methanesulfonate anesthetic solution (20 - 30 min)**
- 2.10.2. Shot of incident light illuminating head region.
- 2.10.3. Talent holding body secure.
- 2.11. Properly identify and focus on the jaw area under the stereomicroscope [1]. Adjust imaging parameters including contrast, brightness, exposure, gain, and white balance for optimal acquisition [2].
- 2.11.1. SCREEN: 2.11.2.mp4 00:10-00:20.
- 2.11.2. SCREEN: 2.11.2.mp4 00:25-00:40.
- 2.12. Then acquire images with a 0.7X stereomicroscope lens to measure and monitor regeneration over successive days [1]. Then, stain regenerated tissues using alcian blue and alizarin red and image them [2].
- 2.12.1. SCREEN: 2.12.1.mp4 00:13-00:25.
- 2.12.2. SCREEN: 2.12.2.mp4 00:13-00:25.

Results

3. Results

- 3.1. A 100% survival rate was achieved in all 37 axolotls that underwent complete transverse mandibular amputation for the first time [1]. The animals showed no changes in swimming, foraging, or jaw movement behaviors following surgery, indicating the safety of the surgical technique [2].
 - 3.1.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the bar labeled “full cross-sectional” showing 100% on the vertical axis.*
 - 3.1.2. LAB MEDIA: Figure 2B.
- 3.2. Macroscopic observation prior to amputation revealed the normal appearance of the mandibular tissues [1]. Immediately after amputation, the remaining soft tissue showed hemostatic response and retraction, resulting in a concavity at the amputation site [2].
 - 3.2.1. LAB MEDIA: Figure 3B.
 - 3.2.2. LAB MEDIA: Figure 3C. *Video editor: Please highlight the area pointed at by the red arrow*
- 3.3. In cases with exposed bone tissue post-amputation, bone regularization was performed, improving wound closure and soft tissue coverage of the amputation site [1].
 - 3.3.1. LAB MEDIA: Figure 3D.
- 3.4. At 180 days post-amputation, the jaw had fully regenerated to a size similar to its original pre-amputation state [1]. The regenerated structure at 180 days included both soft tissue and skeletal elements [2].
 - 3.4.1. LAB MEDIA: Figure 3E
 - 3.4.2. LAB MEDIA: Figure 3F. *Video editor: Highlight the red arrow pointing to regenerated soft tissue and the yellow arrow pointing to regenerated skeletal structures.*

1. Transverse

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

IPA: /'trænzˌvɜːs/, /trænz'vɜːs/

Phonetic Spelling: tranz·vurs

2. Amputation
Pronunciation link: <https://www.merriam-webster.com/dictionary/amputation>
IPA: /,æm.pjə'teɪ.fən/
Phonetic Spelling: am·pyuh·tay·shuhn
3. Distal
Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>
IPA: /'dɪs.təl/
Phonetic Spelling: dis·tuhl
4. Axolotl
Pronunciation link: <https://www.merriam-webster.com/dictionary/axolotl>
IPA: /'æk.sə'lɑ:.təl/
Phonetic Spelling: ak·suh·laa·tuhl
5. Mandible
Pronunciation link: <https://www.merriam-webster.com/dictionary/mandible>
IPA: /'mæn.də.bəl/
Phonetic Spelling: man·duh·buhl
6. Regenerative
Pronunciation link: <https://www.merriam-webster.com/dictionary/regenerative>
IPA: /rɪ'dʒɛn.ə.rə.tɪv/
Phonetic Spelling: ruh·jen·uh·ruh·tiv
7. Imaging
Pronunciation link: <https://www.merriam-webster.com/dictionary/imaging>
IPA: /'ɪm.ɪ.dʒɪŋ/
Phonetic Spelling: im·ij·ing
8. Holtfreter's
Pronunciation link: No confirmed link found
IPA: /'hoʊlt fri:.tərz/
Phonetic Spelling: hohlt·free·terz
9. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /ə'nes.θə.taɪzd/
Phonetic Spelling: uh·nes·thuh·tyzd
10. Supine
Pronunciation link: <https://www.merriam-webster.com/dictionary/supine>
IPA: /'su:.paɪn/
Phonetic Spelling: soo·pine
11. Calliper
Pronunciation link: <https://www.merriam-webster.com/dictionary/caliper>
IPA: /'kæl.ə.pə/
Phonetic Spelling: kal·uh·per
12. Proximal
Pronunciation link: <https://www.merriam-webster.com/dictionary/proximal>
IPA: /'prɔ:k.sɪ.məl/
Phonetic Spelling: prok·suh·muhl

13. Symphysis
 Pronunciation link: <https://www.merriam-webster.com/dictionary/symphysis>
 IPA: /'sɪm.fə.sɪs/
 Phonetic Spelling: sim·fuh·suhs
14. Trajectory
 Pronunciation link: <https://www.merriam-webster.com/dictionary/trajectory>
 IPA: /trə'dʒɛk.tə.i/
 Phonetic Spelling: truh·jek·tuh·ree
15. Anatomical
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anatomical>
 IPA: /,æn.ə'tɑ:.mɪ.kəl/
 Phonetic Spelling: an·uh·taa·mi·kuhl
16. Spatula
 Pronunciation link: <https://www.merriam-webster.com/dictionary/spatula>
 IPA: /'spætʃ.ə.lə/
 Phonetic Spelling: spach·uh·luh
17. Meckel's
 Pronunciation link: No confirmed link found
 IPA: /'mɛk.əlz/
 Phonetic Spelling: mek·uhlz
18. Cartilage
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cartilage>
 IPA: /'kɑ:r.təl.ɪdʒ/
 Phonetic Spelling: kar·tuh·lij
19. Transversely
 Pronunciation link: <https://www.merriam-webster.com/dictionary/transversely>
 IPA: /trænz'vɜ:s.li/
 Phonetic Spelling: tranz·vurs·lee
20. Paraformaldehyde
 Pronunciation link: <https://www.merriam-webster.com/dictionary/paraformaldehyde>
 IPA: /,pær.ə.fɔ:r'mæl.də'haɪd/
 Phonetic Spelling: pair·uh·for·mal·duh·hyde
21. Trizol
 Pronunciation link: No confirmed link found
 IPA: /'traɪ.zəl/
 Phonetic Spelling: try·zol
22. Nucleic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleic>
 IPA: /nu:'kli:.ɪk/, /nju:'kli:.ɪk/
 Phonetic Spelling: noo·klee·ik
23. Petri
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>
 IPA: /'pi:.tri/
 Phonetic Spelling: pee·tree

24. Stereomicroscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/stereomicroscope>
IPA: /,ster.i.oo'maɪ.krə,skoʊp/
Phonetic Spelling: ster·ee·oh·my·kruh·skohp
25. Sulfamerazine
Pronunciation link: <https://www.merriam-webster.com/dictionary/sulfamerazine>
IPA: /,sʌl.fə'mer.ə,zi:n/
Phonetic Spelling: sul·fuh·mer·uh·zeen
26. Postoperative
Pronunciation link: <https://www.merriam-webster.com/dictionary/postoperative>
IPA: /,poust'ɑ:.pə.ə.tɪv/
Phonetic Spelling: pohst·op·uh·ruh·tiv
27. Tactile
Pronunciation link: <https://www.merriam-webster.com/dictionary/tactile>
IPA: /'tæk.təl/, /'tæk.taɪl/
Phonetic Spelling: tak·tuhl
28. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /,æk.wə'zɪf.ən/
Phonetic Spelling: ak·wuh·zish·uhn
29. Incident
Pronunciation link: <https://www.merriam-webster.com/dictionary/incident>
IPA: /'ɪn.sə.dənt/
Phonetic Spelling: in·suh·duhnt
30. Parameters
Pronunciation link: <https://www.merriam-webster.com/dictionary/parameter>
IPA: /pə'ræm.ə.tə/
Phonetic Spelling: puh·ram·uh·ter
31. Alcian
Pronunciation link: No confirmed link found
IPA: /'æl.si.ən/
Phonetic Spelling: al·see·uhn
32. Alizarin
Pronunciation link: <https://www.merriam-webster.com/dictionary/alizarin>
IPA: /ə'lɪz.ə.rɪn/
Phonetic Spelling: uh·liz·uh·rin
33. Macroscopic
Pronunciation link: <https://www.merriam-webster.com/dictionary/macroscopic>
IPA: /,mæk.rə'ska:.pɪk/
Phonetic Spelling: mak·ruh·skop·ik
34. Hemostatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hemostatic>
IPA: /,hi:.mə'stæt.ɪk/
Phonetic Spelling: hee·muh·stat·ik

35. Concavity

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

IPA: /kɑːnˈkæv.ə.ti/

Phonetic Spelling: kon·kav·uh·tee

36. Regularization

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

IPA: /ˌrɛɡ.jə.lə.əˈzeɪ.ʃən/

Phonetic Spelling: reg·yuh·luh·ruh·zay·shuhn

37. Skeletal

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

IPA: /ˈskɛl.ə.təl/

Phonetic Spelling: skel·uh·tuhl