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Title: Development of a Rat Caudal Interbody Fusion Model: A Pilot Feasibility Study

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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? **No**

- 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: 13

Number of Shots: 32

Interviews

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

INTRODUCTION:

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

- 1.1. **Taku Ikegami:** Our research focuses on examining intracage bone evaluation using a rat caudal interbody fusion model with cage and plate fixation.
 - 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.~~

- 1.1. **Taku Ikegami:** This protocol overcomes existing rat model limitations by enabling minimally invasive mechanistic interbody fusion analysis and direct bone formation evaluation.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.2. **Taku Ikegami:** This approach allows quantitative histomorphometric measurement of bone volume, maturity, and osteoconduction patterns within an interbody cage.
 - 1.2.1. 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?~~

- 1.3. **Taku Ikegami:** Beyond histology, this protocol supports micro-CT imaging, biochemical bone turnover assays, and gene expression analysis.
 - 1.3.1. 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.~~

- 1.4. **Taku Ikegami:** Future studies can apply this model to evaluate novel cage materials and investigate spinal fusion under osteoporosis or diabetes condition.
 - 1.4.1. 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 Jikei University

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Surgical Procedure

Demonstrator: Taku Ikegami

- 2.1. To begin, gather the internal fixation materials [1]. Using a 25-gauge needle [2], administer the anesthetic solution [3] subcutaneously into the dorsum of the male Sprague Dawley rat [4].
 - 2.1.1. WIDE: Talent walking into the room with a tray of internal fixation materials.
***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent holding a 25g needle with the rat on the workbench placed in a restrainer.
 - 2.1.3. TEXT ON A PLAIN BACKGROUND:
Anesthesia:
Medetomidine - 0.15 mg/kg
Midazolam - 2 mg/kg
Butorphanol - 2.5 mg/kg
 - 2.1.4. Talent administering the anesthetic mixture subcutaneously into the dorsum of the rat using a 25-gauge needle.
- 2.2. Assess the adequate surgical plane of anesthesia every 15 minutes by confirming the loss of the pedal withdrawal reflex [1], maintaining pink mucous membranes [2], and monitoring the respiratory rate [3].
 - 2.2.1. Close-up of the talent pinching the rat's paw with forceps.
 - 2.2.2. Close-up of the rat's oral cavity as the talent lifts the lip to visually confirm pink mucous membranes.
 - 2.2.3. Close-up of the rat's thoracic region showing visible chest rise and fall.
- 2.3. Place the animal in the prone position [1]. Disinfect the entire tail using an alternating scrub protocol [2].
 - 2.3.1. Talent positioning the animal prone on the surgical surface.
 - 2.3.2. Close-up of the talent scrubbing the entire tail using gauze soaked in 70 percent ethanol, followed by povidone-iodine scrub, and finishing with 70 percent ethanol.
- 2.4. Identify the target vertebrae by performing manual palpation starting from the sacrum distally [1]. Identify the first mobile joint as the sacrum first caudal vertebra

- junction [2] and designate the immediately distal segment as the second caudal vertebra to define the second to third caudal vertebra target site [3].
- 2.4.1. Close-up of the talent palpating the tail starting from the sacrum and moving distally
 - 2.4.2. Close-up of the first mobile joint at the sacrum to Co1 junction.
 - 2.4.3. Close-up of the talent marking the Co2 to Co3 target region on the tail.
- 2.5. Make a longitudinal incision of approximately 2-centimeter on the dorsal aspect spanning from the second to the third caudal vertebrae [1]. Expose the posterior surface of each vertebra [2].
- 2.5.1. Talent making a longitudinal incision on the dorsal aspect of the tail between the second and third caudal vertebrae.
 - 2.5.2. Shot of exposing the posterior surface of each vertebra.
- 2.6. Identify the dorsal caudal vein and avoid damaging it [1]. Perform blunt dissection to carefully trim and remove the surrounding soft tissue [2], including the paravertebral muscle and fascia, from the posterior bony surface [3].
- 2.6.1. Talent pointing out the dorsal caudal vein during exposure.
 - 2.6.2. Close-up of the blunt dissection being performed.
 - 2.6.3. Close-up of the paravertebral muscle and fascia being removed from the posterior bony surface.
- 2.7. Using a scalpel, incise the annulus fibrosus between the second and third caudal or C2 (C-two) and C3 (C-three) vertebrae [1]. Using a curette and small forceps, remove the annulus fibrosus and nucleus pulposus via curettage under high-power surgical magnification of at least 3.5 times [2].
- 2.7.1. Close-up of the scalpel incising the annulus fibrosus.
 - 2.7.2. Close-up of the annulus fibrosus and nucleus pulposus being removed using a curette and small forceps.
- 2.8. Confirm complete removal by observing punctate bleeding [1] and absence of residual soft tissue [2].
- 2.8.1. Close-up of the intervertebral space showing punctate bleeding
 - 2.8.2. Close-up of a clean endplate surface without residual tissue.
- 2.9. Using a curette, assess the endplate preparation by tactile palpation [1]. Sweep the tip across the surface to confirm that no eroded region exceeds 25 percent of the total surface area [2]. Probe the surface to ensure there is no penetration into the cancellous bone deeper than 0.5 millimeters [3].
- 2.9.1. Close-up of curette tip being positioned onto the endplate surface.
 - 2.9.2. Close-up of curette tip being swept across the endplate.
 - 2.9.3. Close-up of endplate being probed with the curette tip. **Author's NOTE: 2.9.1, 2.9.2, and 2.9.3 are combined in one shot.**

- 2.10. Insert a titanium cage measuring 2 by 2 millimeters in cross-section and 2 millimeters in height into the intervertebral space until a physical stop is reached [1]. Confirm full-surface contact with both endplates visually to create a firm interference fit [2].
 - 2.10.1. Close-up of the talent inserting the titanium cage into the intervertebral space.
 - 2.10.2. Close-up showing the cage fully seated between the endplates.

3. Fixation and Wound Closure

- 3.1. Using an electric handheld router operating at 7800 rpm equipped with a 2-millimeter spherical file-type burr [1], flatten the bone surface around the designated hole sites in the C2 and C3 vertebrae [2].
 - 3.1.1. Close-up shot of the electric handheld router with the 2 millimeter spherical file-type burr attached
 - 3.1.2. Close-up of the talent flattening the bone surface around the designated hole sites with burr.
- 3.2. With a hand drill equipped with a 1.2-millimeter diameter drill bit, drill holes individually at intervals corresponding to the screw holes of the plastic internal fixation plate [1]. Preload two 6-millimeter stainless-steel screws into the holes of the plastic plate [2].
 - 3.2.1. Close-up of the hand drill creating holes in the vertebrae aligned with the plate configuration.
 - 3.2.2. Close-up of the talent positioning and inserting the screws into the plate prior to fixation.
- 3.3. Insert the screws at a 90-degree angle to a standardized depth of 5 millimeters with a 12-millimeter center-to-center spacing [1]. Using 4-0 (oh) nylon sutures, close the skin incision [2]. Disinfect the tail again with 70 percent ethanol to complete the procedure [3]. ~~After post-operative management and euthanasia, perform radiographic and histological analysis [4].~~
 - 3.3.1. Close-up of the screws being inserted perpendicular into the vertebrae with consistent spacing between them.
 - 3.3.2. Close-up of the talent suturing the skin incision with 4-0 nylon sutures.
 - 3.3.3. Close-up of the tail being wiped with gauze soaked in 70 percent ethanol.
 - 3.3.4. ~~Representative shot of the talent placing the specimen under microscope.~~ **Author's NOTE: Remove it.**

Results

4. Results

- 4.1. During postoperative follow-up, body weight steadily increased from 221.8 grams [1] to 346.8 grams up to 12 weeks [2], indicating physiological homeostasis and indirectly supporting the absence of severe chronic distress [3].
 - 4.1.1. LAB MEDIA: Table 1. *Video editor: Highlight the value 221.8 g at surgery.*
 - 4.1.2. LAB MEDIA: Table 1. *Video editor: Highlight the value 346.8 g at 12 w.*
 - 4.1.3. LAB MEDIA: Table 1.
- 4.2. Wound healing was achieved 4 weeks postoperatively [1], with spontaneous suture extrusion [2] and no signs of infection observed for 12 weeks [3].
 - 4.2.1. LAB MEDIA: Figure 3A and 3B. *Video editor: Emphasize 3B*
 - 4.2.2. LAB MEDIA: Figure 3C.
 - 4.2.3. LAB MEDIA: Figure 3D.
- 4.3. Sequential radiological assessment using micro-computed tomography confirmed no cage loosening or migration between 1 and 12 weeks postoperatively [1].
 - 4.3.1. LAB MEDIA: Figure 4A–4F. *Video editor: Show the series of images labeled A to F and emphasize the block in the middle across all panels*
- 4.4. Digital radiography at 12 weeks demonstrated no evidence of screw loosening or migration [1].
 - 4.4.1. LAB MEDIA: Figure 4G, 4H. *Video editor: Show panels G and H, and point to the thin, bright, straight rod-like shapes on both sides of the central block.*
- 4.5. At 4 weeks, new bone formation extended from the extra-cage vertebral endplates into the intervertebral space [1].
 - 4.5.1. LAB MEDIA: Figure 4D. *Video editor: Emphasize the arrow and the short thin black line the arrow is pointing at.*
- 4.6. By 12 weeks, the cranial and caudal vertebral bodies were completely bridged by new bone, both inside and outside the cage [1].
 - 4.6.1. LAB MEDIA: Figure 4E, 4F. *Video editor: Emphasize the arrows and the solid white area in the middle block.*

- 4.7. Bright-field microscopy demonstrated complete bony bridging connecting the cranial and caudal sides of the cage at 12 weeks postoperatively [1].
 - 4.7.1. LAB MEDIA: Figure 5A. *Video editor: Emphasize the light purple area that is in between the top and bottom black block.*
- 4.8. Fluorescence microscopy revealed extensive labeling in the caudal region [1] and minimal labeling in the cranial region [2].
 - 4.8.1. LAB MEDIA: Figure 5B. *Video editor: In the space between top and bottom black blocks, circle and highlight the right half (green glowing area above the label caudal)*
 - 4.8.2. LAB MEDIA: Figure 5B. *Video editor: In the space between top and bottom black blocks, circle and highlight the left half area (with little green glow above the label cranial).*
- 4.9. Bone Volume fraction within the cage reached approximately 73.9 percent in the cranial region [1] and 70.5 percent in the caudal region, indicating extensive osseous filling [2].
 - 4.9.1. LAB MEDIA: Table 2. *Video editor: Highlight the value 73.8916% in the row cranial under BV/TV.*
 - 4.9.2. LAB MEDIA: Table 2. *Video editor: Highlight the value 70.4598% in the row caudal under BV/TV.*
- 4.10. Lamellar bone formation was more prominent in the cranial region [1] than in the caudal region [2].
 - 4.10.1. LAB MEDIA: Figure 5C–E. *Video editor: In figure 5C, emphasize the white dotted box D labeled as cranial and emphasize the figure 5D.*
 - 4.10.2. LAB MEDIA: Figure 5C–E. *Video editor: In figure 5C, emphasize the white dotted box E labeled as caudal and emphasize the figure 5E.*

Pronunciation guides:

1. Subcutaneously
 Pronunciation link: <https://www.merriam-webster.com/dictionary/subcutaneously>
 IPA: /ˌsʌb.kjuːˈteɪ.ni.əs.li/
 Phonetic Spelling: sub·kyoo·tay·nee·uhs·lee
2. Dorsum
 Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsum>
 IPA: /ˈdɔːr.səm/
 Phonetic Spelling: dor·suhm
3. Sprague
 Pronunciation link: <https://www.merriam-webster.com/dictionary/Sprague>

- IPA: /spreɪɡ/
Phonetic Spelling: sprayg
4. Dawley
Pronunciation link: No confirmed link found
IPA: /'dɔː.li/
Phonetic Spelling: daw·lee
5. Medetomidine
Pronunciation link: <https://www.howtopronounce.com/medetomidine>
IPA: /ˌmɛd.ɪ'toʊ.mɪ.diːn/
Phonetic Spelling: meh·dih·toe·mih·deen
6. Midazolam
Pronunciation link: <https://www.merriam-webster.com/medical/midazolam>
IPA: /mɪ'dæz.ə.læm/
Phonetic Spelling: mih·daz·uh·lam
7. Butorphanol
Pronunciation link: <https://www.merriam-webster.com/medical/butorphanol>
IPA: /bjuː'tɔːr.fə.nɑːl/
Phonetic Spelling: byoo·tor·fuh·nol
8. Mucous
Pronunciation link: <https://www.merriam-webster.com/dictionary/mucous>
IPA: /'mjuː.kəs/
Phonetic Spelling: myoo·kuhs
9. Vertebrae
Pronunciation link: <https://www.merriam-webster.com/dictionary/vertebrae>
IPA: /'vɜː.t̬ə.breɪ/
Phonetic Spelling: vur·tuh·bray
10. Palpation
Pronunciation link: <https://www.merriam-webster.com/dictionary/palpation>
IPA: /pæl'peɪ.ʃən/
Phonetic Spelling: pal·pay·shuhn
11. Sacrum
Pronunciation link: <https://www.merriam-webster.com/dictionary/sacrum>
IPA: /'seɪ.krəm/
Phonetic Spelling: say·kruhm
12. Distally
Pronunciation link: <https://www.merriam-webster.com/dictionary/distal>
IPA: /'dɪs.təl.i/
Phonetic Spelling: dis·tuh·lee
13. Caudal
Pronunciation link: <https://www.merriam-webster.com/dictionary/caudal>
IPA: /'kɔː.dəl/
Phonetic Spelling: kaw·duhl

14. Paravertebral
Pronunciation link: <https://www.howtopronounce.com/paravertebral>
IPA: /ˌpær.əˈvɜː.tə.brəl/
Phonetic Spelling: pair-uh-vur-tuh-bruhl
15. Fascia
Pronunciation link: <https://www.merriam-webster.com/dictionary/fascia>
IPA: /ˈfæʃ.i.ə/, /ˈfeɪ.ʃə/
Phonetic Spelling: fash-ee-uh
16. Annulus
Pronunciation link: <https://www.merriam-webster.com/dictionary/annulus>
IPA: /ˈæn.jə.ləs/
Phonetic Spelling: an-yuh-luhs
17. Fibrosus
Pronunciation link: <https://www.howtopronounce.com/fibrosus>
IPA: /faɪˈbrʊ.səs/
Phonetic Spelling: fye-broh-suhs
18. Curettage
Pronunciation link: <https://www.merriam-webster.com/medical/curettage>
IPA: /ˌkjʊr.əˈtɑːʒ/
Phonetic Spelling: kyoor-uh-taazh
19. Punctate
Pronunciation link: <https://www.merriam-webster.com/medical/punctate>
IPA: /ˈpʌŋk.teɪt/
Phonetic Spelling: punk-tayt
20. Tactile
Pronunciation link: <https://www.merriam-webster.com/dictionary/tactile>
IPA: /ˈtæk.taɪl/, /ˈtæk.təl/
Phonetic Spelling: tak-tyle
21. Eroded
Pronunciation link: <https://www.merriam-webster.com/dictionary/erode>
IPA: /ɪˈroʊ.dɪd/
Phonetic Spelling: ih-roh-did
22. Cancellous
Pronunciation link: <https://www.merriam-webster.com/medical/cancellous>
IPA: /kænˈsel.əs/
Phonetic Spelling: kan-sel-uhs
23. Intervertebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/intervertebral>
IPA: /ˌɪn.təˈvɜː.tə.brəl/
Phonetic Spelling: in-tur-vur-tuh-bruhl
24. Sutures
Pronunciation link: <https://www.merriam-webster.com/dictionary/suture>

IPA: /'su:.tʃəz/

Phonetic Spelling: soo·cherz

25. Homeostasis

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

IPA: /,hoʊ.mi.oʊ'steɪ.sɪs/

Phonetic Spelling: hoh·mee·oh·stay·suhs

26. Cranial

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

IPA: /'kreɪ.ni.əl/

Phonetic Spelling: kray·nee·uhl

27. Osseous

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

IPA: /'ɑ:.si.əs/

Phonetic Spelling: ah·see·uhs

28. Lamellar

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

IPA: /lə'mel.ə/

Phonetic Spelling: luh·mel·er