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Title: A Surgical Method to Establish a Murine Model of Knee Osteoarthritis using a Modified Hulth Procedure

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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? **Yes.**

If **Yes**, can you record movies/images using your own microscope camera?

No.

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Olympus SZ61

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

SCOPE shots: 2.7.1, 2.9.1, 2.9.2, 2.10.1, 2.10.2, 2.10.3

Videographer: Please film the above-mentioned shots using the scope kit

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: 24

Number of Shots: 49 (6 Scope)

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.***

Answers are slightly edited in accordance with the journal guidelines

INTRODUCTION:

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

- 1.1. **Shaokang Zhao:** Our research focuses on the degradation of cartilage caused by arthritis.

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

~~**Describe the research contexts or systems in which this protocol can be applied.**~~

- 1.2. **Shaokang Zhao:** Our protocol is applicable to the study of both the acute and mid-to-late stages of arthritis

- 1.2.1. 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?**~~

- 1.3. **Chunxiao Yang:** In this study, early swelling and effusion are assessed using ultrasound.

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

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

- 1.4. **Shaokang Zhao:** The surgery itself is the most challenging step; performing it swiftly is key.

- 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. **Chunxiao Yang:** Future studies can explore the drug or physical stimulation therapies for arthritis.

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 Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine

Protocol

2. Mouse Surgery Procedure

Demonstrator: Shaokang Zhao

2.1. To begin, position the mouse in a supine position with the knee joints facing upward to ensure stability and proper access to the surgical site [1-TXT].

2.1.1. WIDE: Talent positioning the mouse on its back on the surgical surface with both knee joints facing upward. **TXT: Anesthesia: Isoflurane; Induction: 2 – 3%; Maintenance: 1 – 1.5%**

2.2. Confirm the depth of anesthesia by checking for the absence of reflexes upon pinching a toe [1]. Apply depilatory cream to completely remove the hair around the knee joint [2].

2.2.1. Talent assessing reflex response of the anesthetized mouse upon pinching toe.

2.2.2. Talent applying depilatory cream around the knee joint. **Videographer's NOTE: 2.2.2 and 2.3.1 were recorded in the same continuous shot**

2.3. Now, remove the residual cream using gauze or cotton swabs [1] and disinfect the exposed skin with 75 percent ethanol [2]. Then disinfect with iodine tincture to ensure aseptic conditions [3].

2.3.1. Talent wiping off depilatory cream with gauze or cotton swabs.

2.3.2. Talent applying 75 percent ethanol to the exposed knee skin.

2.3.3. Talent applying iodine tincture to the same area.

2.4. Extend the hind limb and apply traction to tighten the skin [1]. Using a sterile surgical blade, make a 0.5 to 1-centimeter longitudinal incision along the medial aspect of the right knee joint [2].

2.4.1. Talent extending the mouse hind limb and applying traction to the skin. **Videographer's NOTE: 2.4.1 and 2.4.2 were recorded in the same continuous shot**

2.4.2. Talent making a longitudinal incision along the medial aspect of the right knee joint with a sterile surgical blade.

2.5. Then, retract the skin to expose the underlying tissue [1]. Perform blunt dissection of the fascia to fully expose the joint capsule [2] and visualize the patellar ligament [3].

2.5.1. Talent retracting the skin to reveal underlying structures. **Videographer's NOTE:** 2.5.1 and 2.5.2 were recorded in the same continuous shot

2.5.2. Talent performing blunt dissection of the fascia to expose the joint capsule.

2.5.3. Close-up of the exposed joint capsule and visible patellar ligament. **Videographer's NOTE:** 2.5.3 is located in the "Scope" folder

2.6. Using a fine injection needle, probe the fascia overlying the medial collateral ligament [1]. Incise the fascia overlying the medial collateral ligament [2].

2.6.1. Talent probing the fascia over the medial collateral ligament with a fine injection needle. **Videographer's NOTE:** 2.6.1, 2.6.2, and 2.7.1 were recorded in the same continuous shot

2.6.2. Talent incising the fascia over the medial collateral ligament with the same needle.

2.7. With microscissors under magnification, transect the medial collateral ligament [1].

2.7.1. SCOPE: View under magnification of microscissors transecting the medial collateral ligament. **Videographer: Please film the SCOPE shots using the scope kit**

2.8. Using a needle, displace the patellar ligament to expose the intra-articular space [1] and identify the medial meniscus [2].

2.8.1. Talent displacing the patellar ligament with a needle.

2.8.2. Close-up of pointing to the intra-articular space with the medial meniscus visible.

2.9. Now, employ micro-scissors to transect the medial meniscotibial ligament [1]. Gently probe the tissue with a needle to confirm complete detachment and mobility of the meniscus [2].

- 2.9.1. SCOPE: Micro-scissors cutting the medial meniscotibial ligament.
- 2.9.2. SCOPE: Needle gently probing the meniscus to confirm mobility.

2.10. Identify the anterior cruciate ligament [1]. Transect the anterior cruciate ligament while avoiding damage to the posterior cruciate ligament and lateral meniscus [2]. Observe the positive anterior drawer sign post-operatively to confirm joint instability [3].

- 2.10.1. SCOPE: Identification of the anterior cruciate ligament within the joint.
Videographer's NOTE: 2.10.1 and 2.10.2 were recorded in the same continuous shot
- 2.10.2. SCOPE: Transection of the anterior cruciate ligament with adjacent posterior cruciate ligament and lateral meniscus preserved.
- 2.10.3. SCOPE: demonstrating anterior drawer movement of the knee joint indicating instability.

3. Postoperative Procedure and Care

- 3.1. Close the incision with 4-0 (4-oh) nylon sutures [1] and disinfect the surgical site with iodine tincture [2].
 - 3.1.1. Talent suturing the knee incision with 4-0 nylon sutures.
 - 3.1.2. Talent applying iodine tincture to the sutured surgical site.
- 3.2. Place the mouse on a temperature-controlled heating pad until it regains consciousness and exhibits normal breathing and motor activity [1]. Return the fully recovered mouse to its home cage once no signs of distress are observed [2].
 - 3.2.1. Talent placing the mouse on a temperature-controlled heating pad for recovery.
 - 3.2.2. Talent returning the recovered mouse to its home cage.
- 3.3. Monitor suture integrity continuously for 3 to 5 days following surgery [1]. Re-anesthetize the mouse and perform re-suturing if suture breakage is observed [2].
 - 3.3.1. Talent inspecting the sutures on the mouse knee.
 - 3.3.2. *Reuse 3.1.1.*

3.4. Administer intramuscular antibiotics for up to 3 days after surgery to prevent postoperative infection [1].

3.4.1. Talent administering an intramuscular antibiotic injection to the mouse.

3.5. Monitor the surgical site daily for signs of swelling, discharge, or local inflammation [1].

3.5.1. Talent examining the knee surgical sit. **Videographer's NOTE: 3.5.1 is the same as 3.3.1 and can reuse the footage from 3.3.1**

3.6. Assess joint swelling prior to euthanasia [1]. Perform ultrasonography to evaluate structural changes in the knee joint and conduct the rotarod test [2].

3.6.1. Talent positioning the animal for ultrasound on the table.

3.6.2. Talent performing ultrasonography on the mouse knee joint.

3.6.3. ~~Talent placing the mouse on the rotarod apparatus to assess motor performance.~~

Videographer's NOTE: The author suggested removing step 3.6.3 and 4.1.1, VO is merged with the adjacent shots, so please extend the footage

4. Knee Joint Isolation, Sectioning and Processing

Demonstrator: Chunxiao Yang

4.1. After obtaining the knee joint from the mouse, remove the surrounding muscle and fascia while preserving the tibia, fibula, and femur [1-TXT].

4.1.1. ~~Talent dissecting the knee joint from the euthanized animal.~~

4.1.2. Talent carefully removing surrounding muscle and fascia while keeping the tibia, fibula, and femur intact. **TXT: Euthanize the mouse following institutionally approved protocols**

4.2. Fix the dissected joints and decalcify them in 10 percent EDTA solution for at least 30 days, replacing the solution twice per week to ensure complete decalcification [1].

4.2.1. Talent placing the joints into a container with 10 percent EDTA solution for fixation and decalcification.

4.3. Next, embed the decalcified knee joints in paraffin [1] and prepare sagittal sections at a thickness of 4 micrometers [2].

4.3.1. Talent embedding the decalcified knee joints in paraffin.

4.3.2. Talent preparing sagittal sections at 4 micrometers thickness.

4.4. Begin collecting sections once the meniscus is clearly visible [1]. Stain the sections with safranin O and fast green to visualize cartilage and subchondral bone morphology [2]. Evaluate cartilage degradation using the OARSI scoring system [3]. Stain and process the sections with appropriate reagents [4].

4.4.1. Shot of collecting sections.

4.4.2. Talent staining tissue sections with safranin O and fast green.

4.4.3. Talent examining the sample under a microscope.

4.4.4. TEXT ON PLAIN BACKGROUND:

- Deparaffinize: xylene, 3x, 3 min
- Rehydrate: 100%, 90%, 80% ethanol, 2 min each
- Weigert stain: 5 min
- Differentiate: acid differentiating solution, 15 s
- Fast green stain: 5 min
- Differentiate: weak acid solution, 15 s
- Safranin O stain: 4.5 min
- Differentiate: 90% ethanol, 2 to 3 s
- Dehydrate: 100% ethanol, 1 min
- Clear: xylene, 3x, 2 min

4.5. Then, place the sections in a fume hood to allow excess xylene to evaporate [1].

4.5.1. Talent placing stained sections in a fume hood for xylene evaporation.

4.6. Remove the sections from the fume hood [1]. Apply an appropriate amount of neutral balsam to the specimen [2] and carefully cover the specimen with a coverslip [3].

4.6.1. Talent removing the slides from the fume hood.

4.6.2. Talent applying neutral balsam onto the tissue section. Videographer's

NOTE: 4.6.2 and 4.6.3 were recorded in the same continuous shot

4.6.3. Talent placing a coverslip over the specimen.

4.7. Examine the sections under a light microscope for air bubbles [1] and eliminate any visible air bubbles [2].

4.7.1. Talent observing the slide under a light microscope.

4.7.2. Talent gently adjusting the coverslip to remove air bubbles.

4.8. Finally, store the sections in a slide box [1]. Place the slide box in a 60-degrees Celsius oven for 24 hours to ensure complete curing of the neutral balsam [2].

4.8.1. Talent placing the slides into a slide box.

4.8.2. Talent placing the slide box into a 60 degrees Celsius oven.

Results

5. Results

5.1. On postoperative day 7, 3D ultrasound reconstruction revealed a marked increase in joint cavity volume in the model group compared with the sham group [1].

5.1.1. LAB MEDIA: Figure 2A B. *Video editor: Highlight the images and bar labelled "Model,"*

5.2. Rotarod fatigue testing demonstrated a significant reduction in running endurance in the model group compared with the sham group [1].

5.2.1. LAB MEDIA: Figure 2C. *Video editor: Highlight the bar labelled "Model"*

5.3. On postoperative day 28, Safranin O–Fast Green staining showed that the model group exhibited a significantly rougher articular surface with focal and vertical cartilage fissures compared with the sham group [1].

5.3.1. LAB MEDIA: Figure 3A. *Video editor: Zoom in on the pink-red stained regions in "Model" images.*

5.4. The OARSI (*O-A-R-S-I*) scores for cartilage pathology were significantly higher in the model group than in the sham group [1].

5.4.1. LAB MEDIA: Figure 3B. *Video editor: Highlight the bar labelled "Model,"*

- Anesthesia

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

IPA: /ˌæn.əsˈθiː.zə/

Phonetic Spelling: an·uhs·thee·zhuh

- Depilatory

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

IPA: /dɪˈpɪl.əˌtɔːr.i/

Phonetic Spelling: dih·pil·uh·tor·ee

- Aseptic

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

IPA: /ə'sep.tɪk/, /er'sep.tɪk/

Phonetic Spelling: uh·sep·tik; ay·sep·tik

- Patellar

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

IPA: /pə'tel.ər/

Phonetic Spelling: puh·tel·er

- Fascia

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

IPA: /'fæʃ.i.ə/, /'feɪʃ.ə/

Phonetic Spelling: fash·ee·uh; fay·shuh

- Capsule

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

IPA: /'kæp.səl/

Phonetic Spelling: kap·suhl

- Ligament

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

IPA: /'lɪɡ.ə.mənt/

Phonetic Spelling: lig·uh·muhnt

- Collateral

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

IPA: /kə'læt̬.ə.rəl/, /kə'læt̬.rəl/

Phonetic Spelling: kuh·lat·uh·ruhl; kuh·lat·ruhl

- Meniscus

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

IPA: /mə'nɪs.kəs/

Phonetic Spelling: muh·nis·kuhs

- Cruciate

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

IPA: /'kruː.ʃi.ət/

Phonetic Spelling: كرو·shee·ayt

- Intramuscular

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

IPA: /,ɪn.trə'mʌs.kjə.lə/

Phonetic Spelling: in·truh·muhs·kyuh·ler

- Inflammation

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

IPA: /,ɪn.flə'meɪ.ʃən/

Phonetic Spelling: in·fluh·may·shuhn

- Ultrasonography

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

IPA: /,ʌl.trə.sə'nɑː.grə.fi/

Phonetic Spelling: ul·truh·suh·nog·ruh·fee

- Euthanasia

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

IPA: /ˌjuː.θəˈneɪ.ʒi.ə/

Phonetic Spelling: yoo·thuh·nay·zhee·uh

- Decalcify

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

IPA: /diˈkæl.səˌfaɪ/

Phonetic Spelling: dee·kal·suh·fye

- Paraffin

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

IPA: /ˈpær.əˌfɪn/

Phonetic Spelling: pair·uh·fin

- Sagittal

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

IPA: /ˈsædʒ.əˌtəl/

Phonetic Spelling: saj·uh·tl

- Micrometers

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

IPA: /ˈmaɪ.kroʊ.miː.təz/

Phonetic Spelling: my·kroh·mee·terz

- Safranin

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

IPA: /ˈsæf.rəˌnən/

Phonetic Spelling: saf·ruh·nuhn

- Xylene

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

IPA: /ˈzɑɪ.liːn/

Phonetic Spelling: zye·leen