FINAL SCRIPT: APPROVED FOR FILMING

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Title: Anterior Cruciate Ligament Transection and Synovial Fluid Lavage in a Rodent Model to Study Joint Inflammation and Posttraumatic Osteoarthritis

Authors and Affiliations:
Chilan B.G. Leite[1*], Hannah P. Fricke[1*], Serena D. Song[1], Christian Lattermann[1], Julia F. Charles[1,2]

[1]Department of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School
[2]Department of Medicine, Brigham and Women's Hospital, Harvard Medical School

[*]These authors contributed equally

Corresponding Authors:
Chilan B. G. Leite (cbleite@bwh.harvard.edu)
Email Addresses for All Authors:
Chilan B. G. Leite (cbleite@bwh.harvard.edu)
Hannah P. Fricke (hfricke@bwh.harvard.edu)
Serena D. Song (sdsong@bwh.harvard.edu)
Christian Lattermann (clattermann@bwh.harvard.edu)
Julia F. Charles (jfcharles@bwh.harvard.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? **Yes, all done**

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.**

Current Protocol Length
Number of Steps: 18
Number of Shots: 35

Introduction

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

1.1. **Chilan B. G. Leite:** Our research investigates how injury to the anterior cruciate ligament drives inflammation and progression to post-traumatic osteoarthritis, focusing on the inflammatory mechanisms involved.

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

What advantage does your protocol offer compared to other techniques?

1.2. ~~**Hannah P. Fricke**~~ **Serena Song:** Our protocol offers a straightforward, consistent, and reliable method to induce posttraumatic osteoarthritis and monitor the inflammatory response after injury.

1.2.1. 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?

1.3. **Hannah P. Fricke:** The ACLT procedure and methodology for monitoring inflammation provides the experimental setup to further investigate the inflammatory mechanisms contributing to posttraumatic osteoarthritis and possible treatment interventions.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

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

Testimonial Questions (OPTIONAL):

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at Brigham and Women's Hospital

Protocol

2. Surgical Induction of Anterior Cruciate Ligament Transection (ACLT) in Mice for Post-Traumatic Osteoarthritis Modeling

Demonstrator: Chilan B. G. Leite

2.1. To begin, apply eye lubricant to the anesthetized mouse **[1-TXT].** Shave the fur over the knee, covering the front and lateral sides from mid-shin to mid-thigh using small hair clippers **[2]**.

2.1.1. WIDE: Talent applying eye lubricant to the eyes of the anesthetized mouse. **TXT: Anesthesia: Ketamine (100 mg/kg) and xylazine (10 mg/kg) injection (i.p)**

2.1.2. Talent shaving the specified knee region using small clippers.

2.2. Check the mouse for anaesthesia depth by ensuring it is unresponsive to the toe pinch reflex **[1]**. Then disinfect the exposed skin by applying an antibacterial skin cleanser **[2]**.

2.2.1. Talent pinching the mouse's toe and confirming lack of reflex response.

2.2.2. Talent wiping the shaved knee with antibacterial skin cleanser.

2.3. Administer 0.05 milligrams per kilogram of buprenorphine subcutaneously for pre-emptive analgesia **[1]**.

2.3.1. Talent injecting buprenorphine subcutaneously into the mouse.

2.4. Now, position the mouse on its dorsal side under a dissection microscope **[1]**, ensuring the operative knee faces upward within the microscope's viewing field **[2]**. ~~Then cover the mouse with a sterile drape, leaving a small keyhole opening over the surgical site [3].~~

2.4.1. Talent placing the mouse in dorsal position.

2.4.2. Shot of the operative knees are being seen.
AUTHOR'S NOTE: Combined 2.4.1 and 2.4.2 into one shot

~~2.4.3. Talent covering the mouse with the sterile drape and exposing the surgical area.~~
AUTHOR'S NOTE: Shot deleted

2.5. Position the leg with the knee flexed at approximately 90 degrees and the patella facing upward **[1].** Use surgical tape to keep the knee in the flexed position **[2]**. Adjust the microscope to focus on the patella **[3]**.

2.5.1. Shot of the knee being flexed by 90 ° and the patella is facing upward.

2.5.2. Talent securing the knee position in place with tape.

2.5.3. Shot of the patella being focussed on.

2.6. Next, use forceps to pinch the skin over the patella **[1].** Make a small midline longitudinal incision over the knee with surgical scissors **[2]**. Then extend the incision to approximately 1 centimetre and retract the skin to expose the patellar tendon **[3]**.

2.6.1. SCOPE: 2.6.mp4. 00:00-00:05

2.6.2. SCOPE: 2.6.mp4. 00:05-00:10

2.6.3. SCOPE: 2.6.mp4. 00:11-00:17
Added shot: A macro shot of 2.6.1 through 2.6.3 to accompany the microscope clips

2.7. Now, flex the knee to about 120 degrees **[1].** Use the non-dominant hand to keep it flexed and maintain retraction of the skin edges for better visualization of the patellar tendon **[2]**.

2.7.1. Shot of the knee being flexed to 120 °.

2.7.2. Talent flexing the knee and holding the skin back with the non-dominant hand.

2.8. Identify the medial border of the patellar tendon **[1]** and make an incision along it with a number 11 blade, extending from the midpoint to the superior pole of the patella to open the joint capsule **[2-TXT]**.

2.8.1. SCOPE: 2.8.mp4. 00:00-00:05

2.8.2. SCOPE: 2.8.mp4 00:06-00:17
TXT: If bleeding occurs, apply gentle pressure with a sterile cotton swab for 5 - 10 s

2.9. Use blunt-tip forceps to gently grasp the patellar tendon, lifting it upward slightly, and shift it laterally to dislocate the patella and fully expose the knee joint **[1]**.

2.9.1. SCOPE: 2.9.mp4 00:00-00:12

2.10. Locate the infrapatellar fat pad **[1].** Then use blunt-tip forceps to shift its medial portion and expose the anterior cruciate ligament, while preserving fat pad integrity **[2]**.

2.10.1. SCOPE: 2.10.mp4. 00:00-00:04

2.10.2. SCOPE: 2.10.mp4. 00:05-00:15

2.11. While maintaining the knee at 120 degrees flexion, identify the anterior cruciate ligament or ACL, extending from the lateral femoral condyle to the tibial plateau **[1]**.

2.11.1. SCOPE: 2.11.mp4 00:00-00:02
Video Editor: Please freeze frame here

2.12. Then, use microsurgical scissors to transect the ACL, ensuring the surrounding cartilage, meniscus, and ligaments remain intact **[1]**.

2.12.1. SCOPE: 2.12.mp4 00:00-00:04
Video Editor: Please freeze frame here

2.13. Confirm ACL transection by performing an anterior-posterior drawer test. Flex the knee to 90 degrees **[1]**, stabilize the proximal tibia, and gently push the distal femur backward with blunt-tip forceps **[2-TXT]**.

2.13.1. Talent flexing the knee to 90°.

2.13.2. Shot of the proximal tibia being stabilized then the distal femur being pushed back. **TXT: Observe posterior translation of the femur**

2.14. Reposition the patella and patellar tendon by lifting and shifting medially, then close the joint capsule with a single stitch using absorbable 6-0 sutures **[1]**. Close the skin with 2 to 3 stitches using absorbable 6-0 *(six-oh)* sutures **[2-TXT]**.

2.14.1. Talent repositioning the patella and securing the joint capsule with a single suture.
AUTHOR'S NOTE: We split this step into two parts, with the first being repositioning the patella and the second (2.14.1 A) as securing the joint capsule

2.14.2. Talent suturing the skin with 2 to 3 absorbable stitches. **TXT: For sham surgery, perform same procedure without transecting the ACL**

3. Synovial Fluid Harvesting via Knee Joint Lavage in Mice

3.1. Expose the knee joint of a euthanised mouse by making an anterior longitudinal incision over the knee **[1].** Dissect through the skin and underlying tissues to fully reveal the patellar tendon and patella **[2].**

3.1.1. Shot of an incision being made over the knee.

3.1.2. Shot of the skin and underlying tissue being dissected and the patellar tendon and patella being exposed.
AUTHOR'S NOTE: Combined 3.1.1 and 3.1.2 into one shot

3.2. With a number 11 blade, open the joint capsule along the medial side of the patellar ligament, extending from its midpoint to the superior edge of the kneecap **[1]**. Then gently displace the patella laterally to access the joint space, taking care not to damage surrounding structures **[2]**. Flex the knee to approximately 120 degrees to optimize the joint cavity position for synovial fluid collection **[3]**.

3.2.1. Talent carefully incising the medial joint capsule with a No. 11 blade.

3.2.2. Talent moving the patella laterally with forceps, exposing the joint cavity.

3.2.3. Talent adjusting the leg to a 120-degree flexed position.

3.3. Now use a P10 *(P-Ten)* pipette to perform serial lavages of the knee joint with 2.5 microliters of room-temperature PBS and add to a tube prefilled with 100 microliters of PBS for a final volume of 120 microliters **[1-TXT]**.

3.3.1. Talent pipetting 2.5 microliters of PBS into the joint and aspirating fluid repeatedly to complete 8 cycles. **TXT: Perform 8 repetitions; Cumulative knee lavage fluid: 120 µL**

3.4. ~~Immediately add 100 microliters of PBS to 20 microliters of synovial fluid to obtain a final volume of 120 microliters **[1]**.~~ Then centrifuge the diluted knee lavage fluid at 4 degrees Celsius for 5 minutes at 240 *g* to separate the cellular components from the supernatant **[2]**.

~~3.4.1. Talent pipetting 100 microliters of PBS into a tube containing the 20 microliters of synovial fluid.~~
AUTHOR'S NOTE: We start this process with 100 microliters of PBS already in the tube, and then we add the lavage fluid (step 3.3.1) so this step is omitted

3.4.2. Talent placing the tube in a centrifuge and starting the spin cycle.

Results

4. Results

4.1. Knee size increased significantly at 1-day post-ACLT *(A-C-L-T)* **[1]** before progressively declining back to baseline levels **[2]**.

4.1.1. LAB MEDIA: Figure 7A. *Video editor: Highlight the peak of the line graph at the "1 day" timepoint.*

4.1.2. LAB MEDIA: Figure 7A. *Video editor: Highlight the decline of the red line from "1 day" to "8 weeks".*

4.2. Leukocyte recruitment in synovial fluid increased sharply at 1-day post-ACLT **[1]** and remained elevated through the first week before returning to baseline **[2]**. Interleukin-1 beta levels also spiked significantly at 1-day post-surgery **[3]** and rapidly decreased to near-baseline levels by 1 week **[4]**.

4.2.1. LAB MEDIA: Figure 7B. *Video editor: Highlight the increase in the curve till "1 day"*

4.2.2. LAB MEDIA: Figure 7B. *Video editor: Highlight the increase in the curve between "1 day" to "1 week" then emphasize the decline in the curve till "8 weeks"*

4.2.3. LAB MEDIA: Figure 7C. *Video editor: Highlight the increase in the curve till "1 day"*

4.2.4. LAB MEDIA: Figure 7C. *Video editor: Highlight the decline in the curve from "1 day" to "4 weeks"*

4.3. Both Interleukin-6 and TNF-α *(T-N-F-alpha)* increased significantly on the first day after surgery **[1]** and remained elevated 1-week post-surgery before returning to baseline **[2]**.

4.3.1. LAB MEDIA: Figure 7D and E. *Video editor: Highlight the increase in the curve till "1 day"*

4.3.2. LAB MEDIA: Figure 7D and E. *Video editor: Highlight the curve between "1 day" and "1 week'*

4.4. Matrix metallopeptidase 9 levels increased dramatically at 1 day **[1]** and then declined steadily to baseline by 4 weeks post-injury **[2]**.

4.4.1. LAB MEDIA: Figure 7F. *Video editor: Highlight the increase in the curve till "1 day"*

4.4.2. LAB MEDIA: Figure 7F. *Video editor: Highlight the decline in the curve from "1 day" to "4 weeks"*

4.5. MicroCT analysis revealed significantly higher osteoarthritis scores in ACLT-injured knees compared to controls at 8 weeks post-injury **[1]**. Histological evaluation demonstrated substantial cartilage degradation in ACLT knees versus controls at 8 weeks **[2]**.

4.5.1. LAB MEDIA: Figure 8A and B. *Video editor: Highlight the "ACLT" image in A and column in B*

4.5.2. LAB MEDIA: Figure 8C and D . *Video editor: Highlight the "ACLT" image in C and column in D*

Pronunciation Guide:

1. Anterior Cruciate Ligament

Pronunciation link: https://www.merriam-webster.com/dictionary/anterior%20cruciate%20ligament Merriam-Webster+9Merriam-Webster+9Merriam-Webster+9
IPA (US): /ænˌtɪri·ɚ ˌkruːʃi·ət ˈlɪgəmənt/
Phonetic spelling: an-TEER-ee-er KROO-shee-uht LIH-guh-ment

2. Synovial

Pronunciation link: https://www.merriam-webster.com/dictionary/synovial Merriam-Webster
IPA (US): /saɪˈnoʊvi·əl/
Phonetic spelling: sy-NOH-vee-uhl

3. Osteoarthritis

Pronunciation link: https://www.merriam-webster.com/dictionary/osteoarthritis Merriam-Webster
IPA (US): /ˌɑːstiˌoʊˌɑrˈθraɪtɪs/
Phonetic spelling: oss-tee-oh-ar-THRY-tis

4. Lavage

Pronunciation link: https://www.merriam-webster.com/dictionary/lavage Merriam-Webster
IPA (US): /ləˈväzh/
Phonetic spelling: luh-VAHZH

5. Meniscus

Pronunciation link: https://www.merriam-webster.com/dictionary/meniscus Merriam-Webster
IPA (US): /mə-ˈnɪs-kəs/
Phonetic spelling: muh-NISS-kuhs

6. Interleukin-1 (IL-1)

Pronunciation link: https://www.merriam-webster.com/dictionary/interleukin-1 Merriam-Webster
IPA (US): /ˌɪntərˈlu·kɪn wʌn/
Phonetic spelling: in-ter-LOO-kin-wuhn

7. Cytokine

Pronunciation link: https://www.merriam-webster.com/dictionary/cytokine Merriam-Webster
IPA (US): /ˈsaɪtəˌkaɪn/
Phonetic spelling: SY-tə-kīn

8. Analgesia

Pronunciation link: https://www.merriam-webster.com/dictionary/analgesia
Merriam-Webster
IPA (US): /ˌænəlˈdʒi·ʒə/
Phonetic spelling: an-uhl-JEE-zhuh

9. Centrifuge

Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
Merriam-Webster
IPA (US): /ˈsɛntrəˌfyüj/
Phonetic spelling: SEN-truh-fyoohj

10. Cruciate

Pronunciation link: https://www.merriam-webster.com/dictionary/cruciate
Merriam-Webster
IPA (US): /ˈkru·ʃi·ət/
Phonetic spelling: KROO-shee-uht