

Destabilisation of medial meniscus: a mouse model of osteoarthritis


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This HubLE Method describes the protocol for the surgical destabilisation of the medial meniscus, an experimental mouse model of post-traumatic osteoarthritis.

Materials

1. Inhalation or injectable anaesthesia. [Tip No. 1]
2. Electric clippers
3. Skin disinfectant
4. Sterile gloves and drapes
5. Stereo microscope
6. Analgesia. [Tip No. 2]
7. Sterile instruments (surgical blade #11, 2 mm vannas spring scissors, blunt-ended forceps, Hoskins notched tip forceps). [Tip No. 3]
8. Sterile cotton buds. [Tip No. 4]
9. Metal clips for wound closure.
10. Heat pad and recovery box.
11. Glass bead sterilizer. [Tip No. 5]

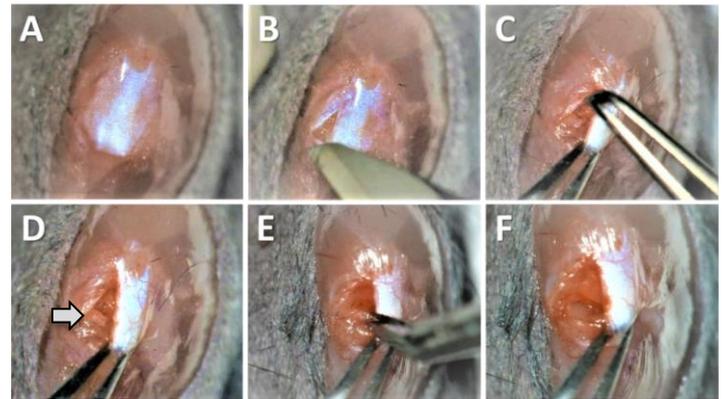


Figure 1: Surgical procedure to identify the medial meniscotibial ligament (MMTL). A) exposure of the patellar ligament. B) Exposure of the infrapatellar fat pad. C) Displacement of the infrapatellar fat pad to allow visualisation of D) MMTL (indicated by an arrow). E) Sectioning of the MMTL with spring scissors. F) Displacement of the meniscus exposing the tibial condyle.

Method

1. The choice of mice age, gender and background depends on the research question you are trying to answer. [Tip No. 6]
2. Induce anaesthesia, shave fur over the right knee and disinfect the exposed skin prior to draping.
3. Administer analgesia by a sub-cutaneous injection or inhaled anaesthesia and place the mouse on a heat pad.
4. Stretch the knee skin to the lateral side and using surgical scissors cut a 5mm longitudinal incision parallel to the distal patellar tendon.
5. To enter the joint capsule, make an incision with a #11 blade along the medial side of the patellar ligament and cut perpendicular towards the medial side. This will expose the infrapatellar fat pad.
6. To visualise the medial meniscotibial ligament (MMTL), move the patellar ligament slightly to the lateral side and the infrapatellar fat slightly to the medial side and up [Tip No. 3].
7. The ligament that attaches the medial meniscus to the anterior tibial plateau is the cranial meniscotibial ligament of the medial meniscus (Fig. 1).
8. Using small curved spring scissors, carefully sever the MMTL, without touching the cartilage at the tibial plateau or femoral condyle.
9. Close the skin incision using 7mm metal clips.
10. For sham surgery, identify the MMTL but do not sever. [Tip No. 7]
11. In between mice, clean instruments and sterilize them. [Tip No. 5]
12. Reverse anaesthesia at the end of the surgery.
13. Replace fluids by a subcutaneous injection and place operated mice in a recovery box.
14. When full consciousness is regained transfer operated mice in a clean cage with fresh bedding and monitor for 72 hours post-operatively. [Tip No. 8]
15. Remove metal clips 7 days post-surgery.
16. Keep mice for 4-52 weeks post-operatively depending on the study design. [Tip No. 9]
17. For evaluation of the model please see Tip No. 10.

Tips

1. For faster anaesthesia induction and recovery it is advised to use inhalable agents (e.g. isoflurane [3.5–4.5% for inducing initial anaesthesia; 1.5–3% for maintaining anaesthesia]) or halothane [3–4% for inducing initial anaesthesia; 1–2% for maintaining anaesthesia]).
2. Buprenorphine (0.05 mg kg⁻¹) or carprofen (5mg kg⁻¹), can be administered subcutaneously.
3. The blunt forceps are used to move the patellar ligament slightly to the side and allow visualisation of the fat pad. The notched tip forceps are not essential but help when moving the fat pad to the side. To cut the ligament you can either use a microblade or spring scissors with short 2mm blades. We find the spring scissors to be more accurate and quicker to cut the ligament without damaging the cartilage.
4. On occasion when moving the fat pad blood vessels rupture. Apply some pressure on the wound with a cotton bud to stop the bleeding.
5. Aseptic technique should be maintained. Some institutions allow glass sterilisers, although the best option is to properly sterilise the surgical instruments in between each surgical procedure. For this it is advisable to have several kits. To maintain aseptic technique it is also advisable that the preparation of the mice (anaesthesia, analgesia and leg shaving) is conducted by an assistant while the surgery is conducted by an operator who does not come into contact with any of the dirty surfaces, hence maintaining a sterile area for the surgery and a “dirty” area for preparation.
6. Most studies use male mice because osteoarthritis severity following DMM is higher than in female mice (1). In males, most studies choose skeletally mature mice, between 10 and 12 weeks old. Evidence shows that osteoarthritis severity at 8-weeks post-operatively, is greatest in 129/SvEv mice, followed by C57BL/10, C57BL/6, FVB/N and DBA/1 (3).
7. Sham operation should be carried out on a separate age-matched group and not on contralateral knee joints.
8. After recovery you should monitor movement of the mice to ensure mobility is optimal. This can be done by simple observation of each mice moving in an empty cage for a minute. Then return to cage with littermates. Mice tend to recover quickly and display no observable changes in their gait or behaviour.
9. Studies have shown that osteoarthritis severity increase through 2-, 4-, 8-, 26- and 52-week post-operation (1, 2, 3). The study design and the choice of duration of the experiment should depend on the research question.
10. Following histological processing osteoarthritis development can be evaluated the OARSI (Osteoarthritis Research Society International) scoring system (4) and the Inflammation/Synovitis scoring System (5).
11. An experienced research can conduct the surgery between 5 and 10 minutes.

References

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